

SHORT REPORT

Pandemic 2009 influenza A(H1N1) virus infection coinciding with invasive pulmonary aspergillosis in neutropenic patients

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SUMMARY

In patients receiving anti-neoplastic chemotherapy, the impact of influenza on the incidence of invasive pulmonary aspergillosis (IPA) remains unknown. We matched data of the Cologne Cohort of Neutropenic Patients (CoCoNut) with records from the Institute for Virology and compared the findings to historical data. During the pandemic, we diagnosed influenza A(H1N1) in five patients with malignancies and febrile neutropenia refractory to antibiotic therapy. Probable IPA was diagnosed in three of these patients on the grounds of typical computed tomography morphology and microbiological results. Three of five patients receiving remission-induction chemotherapy for acute myeloid leukaemia developed aspergillosis although receiving posaconazole prophylaxis. In the 3 years before the influenza pandemic, only 2/77 patients of this group developed infection. Infection with influenza A(H1N1) may increase the risk for invasive aspergillosis in neutropenic patients. Pulmonary aspergillosis is an important additional differential diagnosis in neutropenic influenza patients with pneumonia.

Key words: Aspergillosis, influenza, leukaemia, neutropenia, posaconazole.

It is widely assumed that immunocompromised patients, especially patients with neutropenia after receiving anti-cancer chemotherapy, are at an increased risk of contracting influenza infections with the possibility of severe complications [1]. Although we are not aware of confirmatory data from prospective epidemiological studies allowing the calculation of a hazard ratio, a number of case series have

been published in support of this assumption [2]. A current analysis of the 2009 pandemic showed a considerable morbidity and mortality due to H1N1 infections in patients receiving immunosuppressive medication after solid organ transplantation [3].

Post-influenza pneumonia, usually by bacterial superinfection, is an acknowledged complication of influenza infection. A number of case reports, however, indicated that other opportunistic infections may also be facilitated by seasonal influenza. Lat and colleagues reported two cases of otherwise healthy

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patients developing fatal invasive pulmonary aspergillosis (IPA) secondary to influenza pneumonia with steroid administration during the 2009 pandemic [4]. An earlier analysis of 12 cases of IPA in patients without obvious risk factors revealed that three of them had antecedent or concurrent influenza infections [5].

We performed an analysis of cases from a prospective cohort study to investigate the impact of 2009 pandemic H1N1 influenza on the epidemiology of invasive aspergillosis in high-risk patients.

Clinical data of patients treated on the haematological and oncological wards of Department I for Internal Medicine are prospectively collected in the Cologne Cohort of Neutropenic Patients (CoCoNut) database.

The Institute for Virology of the University Hospital of Cologne provided complete electronic records of positive H1N1 testing of samples and statistics on the influenza A(H1N1) outbreak in Cologne. Cancer patients infected with influenza A(H1N1) during the 2009 pandemic (positive cases were recorded between 26 October and 6 December 2009) were identified and compared to historical records from the same time during 2008–2009. All chest computed tomography (CT) examinations of these patients were re-assessed by a radiologist (C.B.) for infiltrates indicative of IPA. All signs and symptoms of IPA were rated according to the criteria published by the European Organisation for Research and Treatment of Cancer and the Mycoses Study Group (EORTC/MSG) [6]. Influenza infection was assessed by polymerase chain reaction from nasal swabs and bronchoalveolar lavage (BAL) aliquots. Patients were tested for influenza when presenting with symptoms in the emergency or outpatient departments, when fever persisted during neutropenia despite broad-spectrum antibiotic treatment, and after having contact with patients or relatives with influenza infection. BAL specimens of all patients were tested for H1N1. There were no other changes in diagnostic methods or standards during the observational period. The condition of air filters and the aerial load of fungal spores are regularly controlled by the hygiene department.

No construction work was performed close to the haematological/oncological wards during the observational period, and there was no increase in the rate of aerial *Aspergillus* spp. spores during routine controls before and after the observational period. A total of 112 patients with 140 admissions were treated in Department I for Internal Medicine with

anti-cancer chemotherapy and had at least 1 day of neutropenia during the influenza pandemic that struck Cologne between 26 October and 6 December 2009. Of these, five patients were tested positive for influenza A(H1N1). An overview of the patients' characteristics and outcomes is shown in Table 1. Diagnosis was established from nasal swabs (four patients) and/or BAL fluid (four patients). All patients received treatment with 75 mg oral oseltamivir twice daily and made a full recovery from all signs and symptoms of influenza infection. Underlying diseases of the patients were acute myeloid leukaemia (AML, two patients), other haematological malignancy (two patients) and solid tumour (one patient).

Diagnosis of probable IPA according to EORTC/MSG criteria [6] was established in 3/5 influenza patients, including both AML patients. All of these three were profoundly neutropenic at the time of the influenza infection (neutrophil count $< 50/\mu\text{l}$) and did not recover from neutropenia before diagnosis of IPA. All three patients with IPA after influenza recovered from the infection after receiving prolonged antifungal treatment with liposomal amphotericin B and/or voriconazole.

Mycological evidence for IPA was as follows: positive galactomannan samples were obtained from BAL (two patients) and serum (two patients). One patient had *Aspergillus fumigatus* cultured from BAL fluid. Chest CTs were performed for all of the patients, three of which showed infiltrates indicative for IPA (two nodules with surrounding halo and one with a cavity in an area of consolidation).

Two of the three patients with influenza and IPA had AML as an underlying disease. Both had received first line induction chemotherapy and were on regular posaconazole prophylaxis (200 mg oral suspension three times daily) and had acceptable posaconazole serum levels at the time IPA was diagnosed [7].

Including the two patients contracting IPA after influenza, a total of five AML patients received induction chemotherapy between 26 October and 6 December 2009, i.e. an additional three AML patients without diagnosed influenza infection. Of these, one further contracted IPA during neutropenia in spite of posaconazole prophylaxis; however, this patient was not tested for influenza A virus infection. In comparison, in a control group of 77 patients matched for underlying disease and chemotherapy regimen treated between 2006 and 2008, only two patients developed IPA [8]. Thus, the risk ratio for AML patients undergoing induction chemotherapy to

Table 1. Characteristics of five patients with influenza A(H1N1) and persistently febrile neutropenia

Variable	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age, yr	51	23	47	61	80
Sex	Female	Male	Male	Female	Male
Neoplasm	Acute myelogenous leukaemia	Metastasized angiosarcoma	Diffuse large B-cell lymphoma	Multiple myeloma, Salmon–Durie stage III	Acute myelogenous leukaemia
Treatment for neoplasm	High-dose cytarabine and mitoxantrone	Doxorubicin and ifosfamide	High dose oposite	Cyclophosphamide, adriamycin, dexamethasone	High-dose cytarabine and mitoxantrone
Mycological evidence according to EORTC/MSG criteria	Probable	None	Probable	None	Probable
Serum galactomannan	+	–	+	–	–
BAL, galactomannan	–	–	+	Not done	+
BAL, culture result	–	<i>Aspergillus fumigatus</i>	–	Not done	–
H1N1 PCR					
Nasal swab	+	–	+	+	+
BAL fluid	+	+	+	–	+
Days between diagnosis of H1N1 and aspergillosis	4	–	1	–	2
Chest computed tomography	Cavity within area of consolidation	Consolidations and ground-glass opacities	Halo sign	Ground-glass opacity	Halo sign
Treatment					
Osetamivir (75 mg b.i.d.)	Yes	Yes	Yes	Yes	Yes
Antifungal treatment	Yes	No	Yes	No	Yes
Outcome	Survived	Survived	Survived	Survived	Survived

EORTC/MSG, European Organisation for Research and Treatment of Cancer and the Mycoses Study Group; BAL, bronchoalveolar lavage; PCR, polymerase chain reaction.

contract IPA during the influenza A(H1N1) 2009 pandemic was 23.1 (95% confidence interval 4.93–108.16).

Data from our cohort demonstrate a significantly increased incidence of invasive aspergillosis among cancer patients in our department associated with the influenza A(H1N1) 2009 pandemic. More than half of the patients infected by influenza A(H1N1) 2009 developed IPA shortly after the virus were detected, although two of the three patients were on antifungal prophylaxis with posaconazole. While there have been reports of influenza A infection probably predisposing otherwise healthy patients to invasive aspergillosis [4, 5], our observation demonstrates a very high rate of IPA following influenza virus infection in neutropenic patients.

Influenza virus replication leads to a loss of barrier function and reduced ciliary clearance of respiratory tract epithelium [9]. This may allow deeper penetration of fungal conidia upon inhalation and facilitate germination. It is also known that influenza A virus infects and/or debilitates various cells of the immune defence, including polymorphonuclear leukocytes, monocytes, lymphocytes, and alveolar macrophages. Combined with neutropenia following anti-cancer chemotherapy, these effects may predispose the host to fungal infection.

While earlier reports of post-influenza IPA showed a fatality rate of 100% [4, 5, 10, 11], all patients from our cohort survived the infection. It seems possible that the better outcomes of our patients are explained by the higher level of clinical awareness for IPA in the neutropenic host, allowing earlier diagnosis and treatment.

Being a prospective single-centre cohort study, our observations may have been influenced by external factors, e.g. epidemiological shifts that we were unaware of. Given the low number of patients treated for AML with remission-induction chemotherapy at a given time, it was not possible to find sufficient comparators from the same time period. Our controls were therefore selected from a different time period, thus adding to the potential bias mentioned above. However, our observations match expectations from what we know of the pathophysiology of influenza A and *Aspergillus* infections. We suggest performing further analyses using multi-centre observations to confirm the causal relationship of influenza A infection and IPA. Clinicians should maintain a high level of awareness and perform rigorous diagnostic follow-up in neutropenic patients with influenza infection.

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DECLARATION OF INTEREST

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