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Outbreak of influenza virus A/H1N1 in a hospital ward for immunocompromised patients

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In February 2008, five patients were infected with the H1N1 subtype of influenza A virus in one hospital ward for immunocompromised patients at a hospital in North Rhine-Westphalia, Germany. All of these patients had an established haematologic disease and tested positive either for viral RNA or antigen shortly after the beginning of respiratory illness. In three of the patients, influenza virus was repeatedly detected, and four of the patients died in coincidence with the virus infection. Sequencing of the amplified (HA1) haemagglutinin yielded identical nucleotide sequences in isolates from three of the patients, whereas one nucleotide difference was found in the isolate of the fourth patient, resulting in an amino acid substitution (G153R). To investigate the source of infection, the medical staff (n = 104) of the hospital unit was tested and found negative for influenza virus RNA and antigen in pharyngeal lavages. Testing for influenza virus antibodies by immunofluorescence assay revealed that 12 staff members were positive for influenza virus A IgA antibodies. These findings suggest that wild-type influenza virus infections occurred within the medical staff at the same time the patients were infected and that the staff might have contributed to the circulation of virus in the hospital ward.

Introduction

Seasonal influenza is one of the major burdens on public health and is associated with 250,000–500,000 deaths worldwide every year [10]. It is well known that especially the elderly, neonates and patients with underlying chronic diseases are at risk for influenza-associated morbidity and mortality [2, 4]. Several outbreaks of nosocomial influenza have been described in recent years [7, 9, 12]. In these cases, unvaccinated health care workers (HCWs) have been identified as the main source of infection [11]. Annual vaccination against influenza is recommended by almost all national public-health authorities. Nevertheless, the vaccination coverage of HCWs is usually under 40% worldwide [1, 15]. In February 2008, five patients in one hospital ward for immunocompromised patients displayed a sudden onset of respiratory disease. The detection of influenza virus in three of the patients within 4 days alerted the Institute of Virology of the University Hospital of Essen to initiate diagnostic surveillance of the patients in this hospital and to introduce control measures. This report summarizes the findings from the investigation, which suggest that the H1N1 subtype of the influenza A virus was circulating in the hospital ward. Furthermore, our findings indicate that the unvaccinated medical staff might have contributed to the spread of virus between patients.

Materials and methods

Patients

In this investigation, a case of influenza disease was identified if the virus was detected from a respiratory specimen, either by RT-PCR or antigen detection. Individuals were not included if they only presented clinical symptoms of respiratory disease. Antibody responses to influenza virus were not included in the case definition of patients because commercially available assays are not sufficiently reliable, especially in a cohort of leukaemic patients with underlying immunodeficiency.

Detection of influenza viruses

Nasopharyngeal swabs (NP), bronchoalveolar lavages (BAL) or pharyngeal lavages (PL) of patients were tested for viral RNA and influenza virus antigen. For RT-PCR, the viral RNA was extracted from samples (MagNA Pure LC Total Nucleic Acid Isolation Kit, Roche) and assayed by real-time PCR (artus® Influenza LC RT-PCR Kit, Roche) using a LightCycler® Instrument (Roche). Antigen detection

was carried out by two different methods: a rapid antigen detection test (Binax Now Influenza A & B, Binax Inc.) and a cell culture immunofluorescence test (IFT, MDCK cells, Influenza A ? B DFA Kit, Millipore). Patient samples were additionally tested for other respiratory viruses (parainfluenza virus types 1–3, respiratory syncytial virus, adenovirus, enteroviruses) by PCR, antigen assay or virus isolation in tissue cultures. Coinfections with bacterial or fungal pathogens were excluded for all samples. In addition to the specific diagnostics for respiratory disease, patients were generally monitored for infections with microbial and viral pathogens and for reactivation of herpes viruses (herpes simplex virus type 1 and 2, cytomegalovirus, varicella-zoster virus, Epstein–Barr virus) using routine diagnostic methods. For the investigation of the health care workers (HCWs), pharyngeal lavages were tested by the same methods, skipping only the cell-culturebased IFT.

Typing and subtyping of influenza viruses

If they tested positive in either of the assays, specimen were considered positive for influenza virus and were sent to the National Reference Centre for Influenza Virus (Robert-Koch-Institute, Berlin) for further investigation. Typing and subtyping of influenza A viruses was carried out by real-time RT-PCR (TaqMan 7900) as described previously [14] with some modifications (primer and probe sequences available on request). For virus isolation, 200 ll of virus suspension were inoculated onto confluent Madin– Darby canine kidney (MDCK) cells. The cells were maintained in rolling tubes containing serum-free minimum essential medium (Gibco BRL, Life Technologies GmbH, Karlsruhe, Germany) supplemented with 1.25 lg of trypsin per ml (Gibco BRL). The cultures were incubated at 33°C. Cultures were tested continuously for hemagglutination of guinea pig red blood cells (0.5% vol/vol). Hemagglutination-positive cultures were identified using the classical hemagglutination inhibition (HI) procedure. Briefly, specific antisera raised in ferrets were treated with receptor-destroying enzyme. HI tests were carried out with four hemagglutination units of virus and 1.0% (vol/vol) guinea pig red blood cells.

DNA sequencing

PCR products were purified from agarose gels using the JETquick spin column technique (Genomed, Löhne, Germany) according to manufacturer's instructions. Purified PCR products were cycle sequenced in the forward and the reverse directions (primer sequences available on request) in a 3130xl Genetic Analyzer (Applied Biosystems) using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

Phylogenetic analysis

A multiple sequence alignment was compiled from amplified HA sequences using ClustalW in the Bioedit version 7.0.9. Phylogenetic analysis was performed using the PHYLIP version 3.64 package. Sequence distances were calculated, and clustering was done by the neighborjoining algorithm of the PHYLIP program package. The reference strains A/New Caledonia/20/99, A/SolomonIslands/3/2006 and A/Brisbane/59/2007 as well as influenza A/H1N1 viruses circulating in Germany during the 2007/2008 season were included in the phylogenetic analysis. Evaluation of the robustness of the tree was performed by bootstrap analysis carried out with 500 replicates. The tree was plotted using TREEVIEW and was manually edited using the Corel Draw 12 program.

Detection of antibodies

For the investigation of influenza A and B IgA antibodies, a commercially available immunofluorescence assay was used (Fluorimmun-Influenza indirect IFT Kit, Dr. Merck & Coll., Germany). For the time points at which specimen were collected, see ''Results''.

Introduction of control measures

On February 21, 2008, three different patients in the same hospital ward tested positive for influenza virus, indicating the occurrence of an influenza outbreak (Fig. 1). The hospital unit introduced control measures, which were readily implemented on February 22, 2008. Patients testing positive for

influenza virus were cohorted separately from other patients. Individuals presenting symptoms of respiratory disease were placed in private rooms and tested carefully for respiratory viruses. Transfer of patients between units and entrance of visitors was restricted to the absolutely necessary and under conditions of respiratory isolation. Enhanced hand washing and environmental sanitation were carried out. Infected patients were administered either oseltamivir or zanamivir or a combination of both, theywere cared for by vaccinated staff members, and unvaccinated HCWs were offered immediate vaccination. HCWs suffering from respiratory disease were offered treatment with antiviral drugs, and they stayed at home until reconvalescence.

Results

Description of the outbreak

Figure 1 illustrates the detection of influenza virus in five patients. Specimens of patients one, three and four were repeatedly influenza-virus-positive, either by RT-PCR or antigen testing. Patients two, three, four and five died shortly after the last detection of virus, accounting for a case fatality rate of 80%. The median age of the patients was 44.2 years, and all of the patients had an established haematologic disease, having received at least one round of bone marrow transplantation. Patient one recovered from the acute virus infection and displayed prolonged virus shedding, as has been described by others [8, 11]. Excluding this patient, the duration of the outbreak was 22 days (calculated from the first to the last detection of virus in the patients). No new cases emerged after day ten, indicating that the introduction of control measures was effective.

Characterization of the virus isolates

All isolates from the patients were identified to belong to the H1N1 subtype of influenza A by subtype-specific RT-PCR. Sequence analysis of the HA1 region revealed identical nucleotide sequences in the isolates of patients two, four and five, whereas one nucleotide difference was found in the strain from patient one, resulting in an amino acid substitution (G153R, 99% homology). Unfortunately, the isolate from patient three could not be sequenced because of a lack of sample material. These findings indicate that one specific strain of influenza virus was most probably introduced into the hospital ward and spread from one patient to others. A phylogenetic analysis of the HA1 gene (1,000 nucleotides) of the patients' viruses revealed a close relationship to the HA genes of influenza A/H1N1 viruses circulating in Germany during the influenza season 2007/08 and the new reference strain A/Brisbane/59/2007, which was recommended as the H1N1 vaccine strain for 2008/2009 (Fig. 2). The viruses isolated from the patients formed a separate cluster including an influenza virus detected in the German state of Baden-Wuerttemberg during 2007/2008.

The antigenic profile of the virus isolates was analyzed using a set of ferret antisera to current vaccine and reference strains. All of the virus strains reacted well with antiserum to A/Solomon Islands/3/006, the A/H1N1 vaccine strain of the season 2007/2008. However, all isolates were antigenically more closely related to the new reference
strain A/Brisbane/59/2009, which was also the case with the majority of A/H1N1 viruses circulating in Germany during 2007/2008.

Testing of health care workers

To investigate the source of infection, the medical staff (n = 104) of the hospital unit was tested and found negative for influenza RNA and antigen in pharyngeal lavages. The HCWs were additionally tested for IgA antibodies against influenza A and B virus at two different time points. The first serum panel was collected together with the pharyngeal lavages, and the second panel was collected 4 weeks later (March 25 to April 15, 2008). Overall, 12 staff members tested positive for influenza A IgA antibodies, and 13 were positive for influenza B IgA antibodies (Table 1). Of the 104 individuals included in the first panel, 60 were tested again. Of the five individuals who were positive for influenza A IgA antibodies in the first serum panel, four were again positive in the second sample, one person did not receive a second venipuncture and seven staff members who had been IgA negative seroconverted between the first and second tests. With influenza B, among the seven staff members who were positive for IgA antibodies in the second panel, five had already tested positive in the first sample, accounting for two influenza B IgA seroconversions. Of these 11 individuals, six were not

available at the second time point. The detection of IgA antibodies suggest that wild-type influenza virus infections had occurred within the medical staff at the same time the patients were infected and that the HCWs might have contributed to the circulation of virus in the hospital ward.

Discussion

In the present influenza outbreak, the homology of the HA1 sequences found in the affected patients suggests that most probably only one specific strain of influenza virus was introduced into the hospital ward and spread from patient to patient. A circulation of this virus in the hospital unit is more likely than the introduction of identical isolates through different individuals. This hypothesis is confirmed by the fact that patients one and two were placed in the same room prior to the beginning of respiratory disease. The detection of IgA antibodies against influenza A and B in the HCWs suggests that wild-type infections might have occurred among the medical staff at the same time the patients were infected.

The first venipucture of the HCWs was carried out at a time point when the circulation of the virus was detected in the patients by RT-PCR. It has been shown that HCWs can be infected with influenza virus either in their workplace or in the community, and that a portion of them continued to work while ill [16]. In the present case, there were also members of the medical staff who were suffering from respiratory illness at the same time of the disease outbreak. Interestingly, the ratio between positive IgA antibodies against influenza A virus (n = 12) and influenza B virus (n = 13) in both serum panels was 48–52%, reflecting the ratio of circulating influenza subtypes in Germany in the
winter season 2007/08 (48% influenza A: 98% A/H1N1, 2% H3N2; 52% influenza B) [3]. Because all respiratory samples of the HCWs were found negative for influenza RNA and antigen, we were unable to confirm the medical staff to be the original source of infection. There might be different reasons for this. It is known that, even in acute influenza disease, the virus becomes undetectable in the respiratory tract within days after infection [7]. The pharyngeal lavages of the HCWs were collected from February 27 to March 5, 2008, i.e. not less than 9 days after the first detection of virus in the patients. Therefore, PCR and antigen testing could have missed the virus, since it might already have been cleared from the respiratory tract. Another trivial reason might be that the individual who transmitted the virus was among the staff members who were absent from work because of illness. These individuals were excluded from the investigation. Even if there are strong hints of an introduction of the influenza virus through the medical staff, the virus might also have been transmitted to patients by visitors. Another possibility is that the infection of the first patient was communityacquired. This might have happened especially with patient five, who was already receiving mechanical ventilation when he entered the hospital ward. Intubation and other droplet-generating procedures have been shown to increase the risk of transmitting infectious agents to HCWs [5], who might thereafter induce and sustain the circulation of virus between patients.

Phylogenetic analysis revealed that the analysed HA genes of the viruses isolated during the present outbreak were closely related to the HA genes of A/H1N1 viruses circulating in Germany during the influenza season 2007/2008 as well as to the new reference strain A/Brisbane/59/2007. Even if the 2007/2008 A/H1N1 vaccine strain A/Solomon Islands/3/2006 and the newly recommended vaccine strain A/Brisbane/59/2007 are genetically different, both strains are antigenically more similar. Our findings suggest that vaccination with A/Solomon Islands/3/ 2006 would presumably have had a protective effect. It is known that the trivalent inactivated vaccine (TIV) against seasonal influenza has a 70–90% efficacy in healthy adults under 65 years of age [10]. In bone marrow transplant recipients, the response to vaccination is usually lost for at least 6 months after transplantation and gradually returns so that responses are generally intact 2 years after transplantation [6].

The duration of influenza outbreaks varies strongly, ranging between a few days [9] and several months [10, 12, 13], and depends on multiple factors, such as the clientele of patients, the local setting in the hospital, the vaccination rates of HCWs and patients and the introduction of control measures. In this case, the duration of the outbreak was 10 days, suggesting that the introduction of control measures was effective. On the other hand, influenza virus causes an infection with a short incubation period, leading
to an acute respiratory illness and a comparatively short period of virus shedding, at least in non-immunocompromised adults. Therefore, it is not possible to distinguish between the effect of control measures and a self-limitation of the outbreak [8].

In the case of the hospital unit described here, the vaccination coverage of the medical staff and of the patients is not precisely known. However, one can estimate that it was at least not higher than it is worldwide, i.e. less than 40% [1, 15]. This was confirmed by the Department of Occupational Health, which carried out the influenza vaccinations in this hospital. On the basis of our case definition, which only includes individuals for whom influenza virus was detected directly, the extent of the outbreak might have been underestimated. It must also be taken into account that the patients might also have died due to their underlying leukaemic disease and associated complications. In any case, the findings from this influenza outbreak demonstrate the need to have the highest achievable vaccination coverage of HCWs, patients with immunocompromising diseases, and their relatives.

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Tables and Figures

Figure 1 Detection of influenza virus in the five patients involved in the outbreak (positive signs within gray bars). Patients one, three and four were repeatedly found to be influenza virus-positive, either by PCR or antigen testing. All of the patients had an established haematologic disease. All patients but one died shortly after the last detection of virus in respiratory specimens. Patient one recovered from the acute virus infection and displayed prolonged virus shedding. Negative signs indicate negative results of influenza testing

Figure 2 Phylogenetic analysis of the HA1 region of the HA gene of A/H1N1 viruses. This analysis includes A/H1N1 viruses isolated from patients during the outbreak, A/H1N1 viruses circulating in Germany during 2006/2007 (clade 1, clade 2A, group 1 of clade 2B) and during 2007/2008 (group 2 of clade 2B) as well as three reference strains. The strain A/New Caledonia/20/99 was recommended as a vaccine strain until 2006/2007, the strain A/Solomon Islands/3/2006 for the 2007/2008 season, and the strain A/Brisbane/59/2007 was the new A/H1N1 component in the season 2008/2009. Phylogenetic clustering was done by the neighbor-joining algorithm based on a bootstrap analysis with 500 replicates. Isolates from patients are shown in bold type, and reference strains are boxed

Table 1 Serological results of the medical staff of the hospital ward where the influenza outbreak occurred

IgA antibodies positive for	Serum panel 1 27 February to 5 March 2008 ($n = 104$)	Serum panel 2 25 March to 15 April 2008 ($n = 60$)	IgA sero-conversions	Cumulative positive IgA antibodies
Influenza A virus	5	11	7	12
Influenza B virus	11	7	2	13