

The new influenza A (H1N1/09): symptoms, diagnostics, and autopsy results

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Abstract The first infections of the new H1N1 (“swine flu”) virus were announced in April 2009. Soon after, case numbers were observed to be climbing virtually worldwide. In most cases, infections appeared rather mild with typical flu symptoms. In contrast to the seasonal flu, however, cases with vomiting and diarrhea were also reported, and the affected age group seemed to be younger. Sudden and fatal progression affected mostly those under 60 years of age. The autopsy reports of two fatalities with the new influenza are discussed. Multiple postmortem swabs from the nose and throat area determined the H1N1-RNA.

Keywords H1N1 · Influenza · Swine flu · Autopsy

Introduction

In April 2009, the World Health Organization (WHO) announced that in the USA, as well as in Mexico, a new Influenza A (H1N1/09) had been identified, which partially consists of gene segments of different swine influenza viruses. In March, there had already been an outbreak of respiratory infections in Mexico that were associated with this virus. By May, there were 1,124 cases announced

worldwide, and in June, the WHO declared a pandemic [8, 13]. Subsequently, a steadily increasing number of infections occurred in Germany. During the last week of November, the weekly report from the Robert Koch Institute (RKI) reported a decreasing number of cases for the first time [6]. Since then, the number of new infections in most European countries has steadily declined.

By December 22, 2009, a total of 209,885 cases of diagnosed infection with the new influenza virus were reported. Of these, 132 cases were fatal [7].

Most showed signs of a mild clinical course of infection. Typical flu symptoms include a sudden infection with fever ($>38^{\circ}\text{C}$), dry and deep coughing, general malaise, as well as muscle and limb pain. In contrast to the seasonal flu, vomiting or diarrhea are characteristic for the new influenza, either alone, or in addition to the typical symptoms [9]. Out of a total of 411 patients testing positive for the new influenza, Winzer et al. identified nausea and/or vomiting in 3.4% of cases and diarrhea in 1.7% of cases. Furthermore, a portion of the patients showed no sign of fever [14].

Given the high number of cases, the reporting requirements were changed on November 14, 2009. At the beginning of the pandemic, every suspected case was required to be registered by the public health department. However, since the change, only cases of death, as well as laboratory-confirmed infections, are registered [2].

The cases of death registered by the RKI, like in other countries, occurred particularly in people at high risk. Deadly or serious infections requiring hospital care occur primarily in children under 4 years of age, pregnant women, or in persons with chronic preexisting conditions, such as lung infection, cardiovascular disease, diabetes mellitus, renal disease, or a suppressed immune system. Of the 126 registered cases of death that presented with indications of preexisting conditions, a risk factor could

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be determined in 111 (88%) of the cases. Eighty-three percent of those who died were under 60 years of age [8].

In contrast, up to 90% of deaths from the seasonal flu occur in older populations [8].

Case 1

Medical history

A 31-year-old female nurse was found dead by her parents in her living room.

Approximately 5 days previously, she has been declared sick by her doctor with suspicion of a flu-like illness (no diagnostic followed). According to her doctor, she presented with fever, coughing, and limb pain. A few days later, she also suffered from diarrhea. After her parents tried to reach her 1 day several times by telephone, they drove to her apartment in the evening. The deceased was found in her living room with the television on. The back, upper body, and legs were covered with a blanket. White foam was discovered on the deceased's mouth and a bucket of vomit was found next to the sofa. Furthermore, several medications (e.g., Paracetamol, Imodium®) appropriate for the treatment of the flu and/or diarrheal disease were found in the living room.

According to her doctor, there were no known serious preexisting conditions.

Autopsy results

The autopsy showed a strong reddening of the membranes in the respiratory tract. Furthermore, a copious white foam secretion was found to reach to the periphery of the respiratory tract. The lung tissue showed high-grade edematous and demonstrated elevated fragility. Correspondingly, unspecific signs of general inflammation were found, such as postmortem clots and a spodogenous spleen. The lack of a preexisting condition could be confirmed by the autopsy.

Histology

The microscopic examination showed a lung edema as well as pneumonia with prevalent monolymphcytic infiltrates in the interstitium. Granulocytic infiltrates were found in the alveoli as a sign of a beginning super infection (Fig. 1). The remaining organs appeared normal.

Microbiological examination

A swab of the oral cavity, the nose, and throat area, as well as five swabs of the trachea, were taken during the autopsy, which was conducted approximately 24 h after death. The

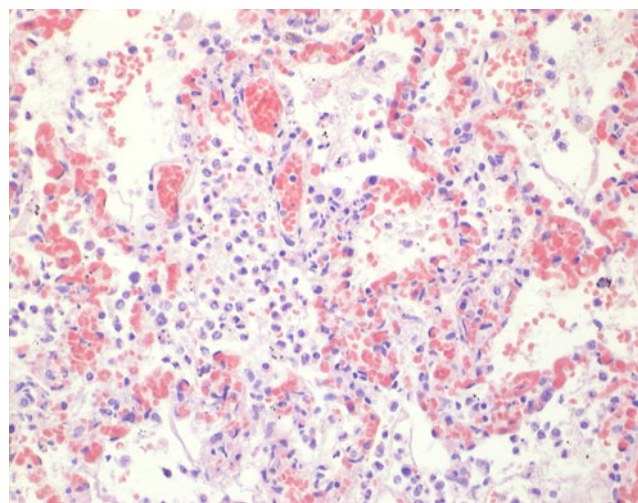


Fig. 1 Lungs (HE): Pneumonia with prevalent monolymphcytic infiltrates in the interstitium, granulocytic infiltrate, and lung edema

swabs of the trachea were stored at 4°C and forwarded to Microbiology Department every 24 h. The H1N1 virus was detectable in the polymerase chain reaction (PCR) in all examinations. Two real-time RT-PCR assays for influenza A(H1N1)v virus targeting the hemagglutinin and matrix gene were performed like described by Panning et al. [5].

Case 2

Medical history

At the end of November 2009, despite intensive medical measures, a 13-year-old girl died in the pediatric clinic of an exudative pericarditis, with suspicion of myocarditis resulting from a highly feverish infection that lasted for several days. Three days prior, the girl complained of throat pain and a headache but, nevertheless, did not visit the doctor and continued to go to school. After she collapsed at home on the fourth day and her condition deteriorated, an emergency doctor was contacted. She was placed under resuscitation in the pediatric clinic with outbreaks of sweat, cold extremities, blue lips, and shallow breathing. She passed away there a short time later. Due to her previous good health and the unclear genesis of the fluid in the pericardium, an unexplained death was documented and a court-ordered autopsy was conducted. A previously taken swab of the nose and throat area was positive for H1N1 ("swine flu").

Autopsy results

Fitting with a viral infection, the autopsy showed tracheitis, bronchitis (i.e., a blazing red respiratory tract with speckled

hemorrhaging), marked hemorrhagic pneumonia, as well as general swelling of the lymph nodes.

Upon opening of the pericardium, 70 ml of clear amber-colored liquid emptied out. The inner surface of the pericardium showed an augmented vessel design, as well as small patch-shaped hemorrhaging on the top of the heart. The heart muscle appeared inhomogeneous, with isolated, lightly sunken in areas measuring up to 2 mm. Otherwise, preexisting internal conditions could not be found.

Histology

The microscopic examination showed a florid myocarditis in the heart with marked mixed-cell infiltrates—lymphocytes, monocytes, plasma cells, as well as granulocytes (with eosinophilia)—as indication of a super infection. Moreover, typical single-phase necrosis, a powerful interstitial edema, a diffuse cell property, as well as a hyperemia with clear rouleaux formation were found (Fig. 2). The pericardium showed inflammation with infiltrations of lymphocytes, polymorphic leukocytes, and/or granulocytes.

Massive hemorrhages could be detected in the lungs and the trachea, as could prevalent monocytic cell groups with infiltration of the bronchial wall, the tracheal wall, and the interstitium. Bronchial eosinophilia and bacterial beds appeared to be an indicator of the occurring aspiration (Figs. 3 and 4).

The remaining organs, aside from an acute blood stasis, appeared to be normal given the patient's age.

Microbiological examination

Before the autopsy, swabs from the nose were taken every 24 h (2, 3, 4, and 5 days postmortem) and were examined

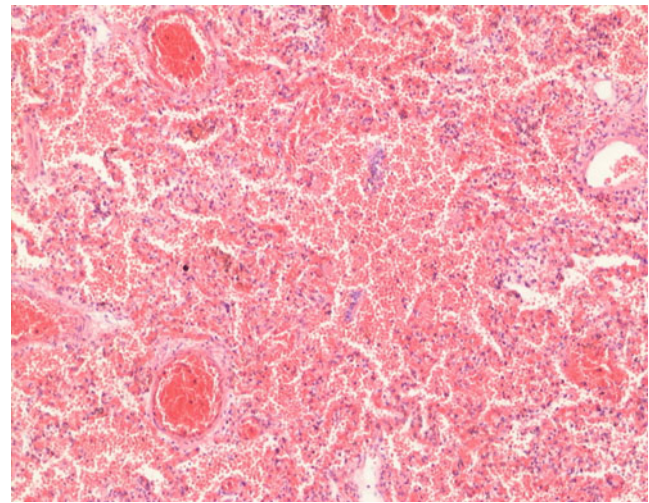


Fig. 3 Lungs (HE): Massive hemorrhaging

by means of PCR. The deceased was cooled at about 4°C for the duration. Postmortem of the lung, the heart, and the brain were also examined. The H1N1 virus was detectable in the PCR in all examinations.

Discussion

In the cases described here, it can be clearly assumed that it was a question of a deadly course of the new influenza. In the case of the 31-year-old woman, a serious pneumonia led to death. In the case of the 13-year-old girl, it was pneumonia, in combination with a myocarditis/pericarditis, that led to death. Based on the examinations conducted (e.g., autopsy, histology, microbiology), chronic preexisting conditions, which belong to the risk factors for severe

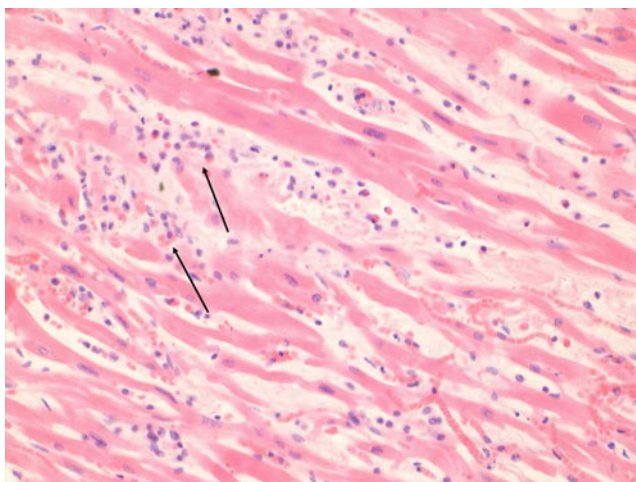


Fig. 2 Heart (HE): Florid myocarditis with mixed-cell infiltrates (arrows), as well as single-phase necrosis, interstitial edema

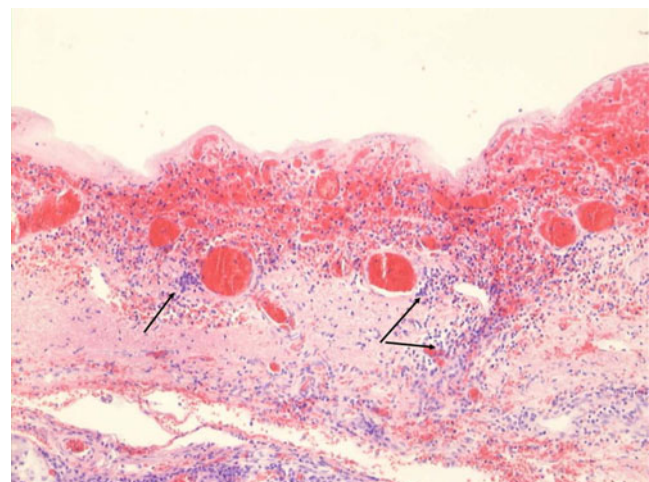


Fig. 4 Trachea (HE): Massive hemorrhaging, prevalent monocytic infiltration of the tracheal wall (arrows), edema

infection, were not detected. As previously reported by the RKI, the young ages of the deceased are striking and rather atypical for the seasonal flu, which mostly leads to death in older age groups.

Maue et al., who analyzed the data from 21 autopsied fatal cases in Brazil, also arrived at similar conclusions. Fifteen patients (72%) were between the ages of 30 and 59 years old. In only three cases (14%) was the age over 60, with the oldest being 68 years. In all cases, the cause of death was damage to the lungs [4].

In the microscopic examination, the lungs appeared edematous in all cases, with varying degrees of parenchymal hemorrhaging. In approximately half of the cases, diffuse alveolar damage with alveolar and interstitial edema, fibrinous exudations, and glassy membranes was detected. In a further six cases, necrotic bronchiolitis was found. In no case did the virus appear to have caused damage to any organ other than the lungs [4].

It has been sufficiently established that viral infections can manifest themselves in the heart in the form of myocarditis and/or pericarditis, though the fulminant course of myocarditis and the direct detection of the pathogen is an extraordinary process. This applies to influenza, measles, mumps, rubella, and polymyositis, among other infections. In approximately 5% of cases, a diagnosis of myocarditis is given postmortem. In most cases, these infections run their course unnoticed, and a diagnosis usually occurs only by chance. For only a few is the infection sudden and deadly, and this is observed primarily in young people, as in the case described here [13].

According to current knowledge, the incubation period and length of infectiousness most likely matches that of the seasonal flu. The incubation period usually amounts to 1 to 2 days but can last up to 4 days. The length of time it takes for the virus to clear the body (i.e., the length of infectiousness) usually amounts to 3–5 days after the first symptoms appear. In rare cases, however, it can also take longer [9]. Currently, the length of postmortem infectiousness is unknown.

To confirm the diagnosis, a swab from the nose and throat area and a PCR to indicate the pathogen are recommended. The application of antigen tests (i.e., “quick tests”) is, as a rule, no longer recommended because they can lead to false-negative results. In the literature, sensitivity has been found to be between 18% and 51% [1, 10]. Additionally, with autopsies, the Royal College of Pathologists recommends the preservation of fresh tissue samples from the lower respiratory tract, the lung tissue, the lower small intestine, the lymph nodes, and the blood [12].

The Institute for Legal Medicine in Hamburg used two different approaches in taking postmortem swabs from the

nose and throat area. In the first approach, swabs were taken during autopsy and afterwards sent to Microbiology Department every 24 h for examination. In the second approach, the swabs were taken consecutively every 24 h before the autopsy. In all cases, the virus could at least be detected in the PCR. Currently, statements about postmortem infectiousness are still not possible because knowledge in regards to infection requires a pathogen count is not given and a quantitative determination is not possible by swabs.

The Centers for Disease Control and Prevention recommend the following existing personal security measures for all autopsies: a gown with an impermeable apron, protective glasses, two pairs of sterile and cut-proof gloves, and mouth protection [3]. The mouth protection should be impermeable to vapor, and the Royal College of Pathologists recommends filter class FFP3 (filter impact >99%) protection masks [12].

Moreover, the autopsy room should be equipped with an appropriate air circulation system and a vacuum system [3, 6]. After an autopsy, the room should be cleaned using the cleaning agents recommended by the RKI. A special hand disinfectant is not necessary [6].

However, as always, immunization counts as one of the most effective preventative measures [9]. The data arising thus far regarding the Pandemrix vaccine document a high immunity, therefore, only a one-time vaccination is necessary [11].

Conclusion

- In the presence of a fever of unknown origin, an influenza infection should be suspected. However, a fever can also be absent.
- Diarrhea and vomiting can be present, either alone or in addition to other symptoms. This does not occur with the seasonal flu.
- The autopsy results are rather unspecific (e.g., pneumonia, lung edema, reddened respiratory tract) and mostly affect the lungs.
- In contrast to the seasonal flu, serious and sudden progression of the infection should be observed for in younger people (<60 years).
- Presently, data regarding postmortem infectiousness are still not available. However, using PCR, the virus is still detectable several days postmortem.
- During autopsy, existing guidelines for personal protection should be followed, and an appropriate mask (FFP3) should be worn.
- The most effective measure for prevention is vaccination.

Conflict of interest The authors declare that they have no conflict of interest.

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