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CASE REPORT

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Fatal influenza A infection with *Staphylococcus aureus* superinfection in a 49-year-old woman presenting as sudden death

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Abstract A fatal case of influenza A infection with *Staphylococcus aureus* superinfection in a previously healthy 49-year-old woman presenting as sudden, unexpected death is reported. Autopsy revealed severe necrotizing tracheobronchitis and hemorrhagic pneumonia. Microscopic examination of the trachea and bronchi showed mucosal necrosis and a dense lympho-monocytic infiltration of all layers. The lungs showed focal hemorrhagic pneumonia. No pathological changes were detectable in the myocardium. Influenza A virus was detected in bronchi and lung samples obtained during autopsy by the polymerase chain reaction (PCR) and bacterial superinfection with *Staphylococcus aureus* was shown by culturing from tracheal, bronchial and pulmonary swabs obtained during autopsy. PCR assays for the detection of Panton-Valentine leukocidin performed from all samples were negative. This case demonstrates the need for an interdisciplinary approach towards an organism-specific diagnosis of potentially infection-related deaths undergoing a medico-legal autopsy. With improved diagnostic possibilities such as PCR and DNA sequencing, forensic pathologists can, in close association with the field of microbiology, make a significant contribution to the detection of highly infectious agents which must be notified to the authorities. This will increase particularly the knowledge about the influence of these agents on sudden, unexpected deaths in outpatients.

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Introduction

Occasionally, the forensic pathologist may be confronted with cases of sudden unexpected death that are the sequel of a fulminant course of a previously undiagnosed infection [1, 2, 3, 4, 5].

Influenza viruses are single-stranded, helical RNA viruses belonging to the family Orthomyxoviridae, and types A and B are the best known. Influenza virus is a globally important respiratory pathogen with a high degree of morbidity and mortality, particularly in risk groups such as elderly persons. Only a few case reports of fatal influenza infections in young patients, most often complicated by influenza-associated myocarditis, have been published during the last decade [6, 7, 8, 9]. A potential differential diagnosis, in both the clinical as well as autopsy setting, is hemorrhagic necrotizing pneumonia caused by Panton-Valentine leukocidin-producing *Staphylococcus aureus* strains. This type of pneumonia, mainly observed in otherwise healthy children and young adults, is often preceded by influenza-like symptoms and has a high lethality rate [10].

Here, we present the pathological features of a fatal case of influenza A infection with *Staphylococcus aureus* superinfection in a young adult that presented as sudden unexpected death.

Case report

After an uneventful 4-day illness with dry cough, shortness of breath and malaise, a 49-year-old woman suddenly collapsed at home after an episode of acute dyspnoe. An emergency physician was called immediately but resuscitation efforts were unsuccessful. Due to the unknown

cause and manner of death, a medico-legal autopsy was performed 30 h postmortem.

The external examination of the body was unremarkable. At autopsy, a hemorrhagic tracheobronchitis was present with trachea and main bronchi partly covered by purulent membranes up to 1.1 cm in diameter and firmly adhering to the mucosal surfaces (Fig. 1). Apart from dilatation of the ventricle and atrium of the right heart and the presence of buffy coat clots within the larger arterial vessels, the gross pathology of the other internal organs was unremarkable.

Microscopic examination of trachea and bronchi showed mucosal necrosis and sloughing as well as a dense lympho-monocytic infiltration of all layers (Fig. 2a, b). The lungs showed focal hemorrhagic pneumonia strictly limited to circumscribed areas corresponding to lobular outlines (Fig. 3). Within such foci of hemorrhagic pneumonia, the alveolar spaces were filled with erythrocytes and the alveolar lining cells were for the most part not detectable. There was neither any microscopic evidence of bacteria nor were any purulent pulmonary abscess formations or hyaline membranes detectable. A considerable congestion of pulmonary vessels accompanied by vascular engorgement and a marked perivascular edema were present in both inflamed and pathologically unaltered pulmonary tissue. No preexisting lung disease could be established by histological means. Apart from a moderate interstitial edema, no pathological changes were detectable in the myocardium. Concerning the presence of inflammatory changes, all internal organs except for the lungs were unremarkable and no microthrombi formation was evident in any organ system by microscopic examination. Neuropathological examination of the brain showed no pathological changes.

Postmortem microbiological cultures of swabs from trachea, bronchi and pulmonary tissue that were obtained during autopsy cultured *Staphylococcus aureus*. Resistance testing showed only the presence of a β-lactamase in this strain, resistance to methicillin could not be detected. PCR assays for the detection of Panton-Valentine leukocidin performed from all samples were negative. Also, all samples were negative for *Haemophilus influenzae*.

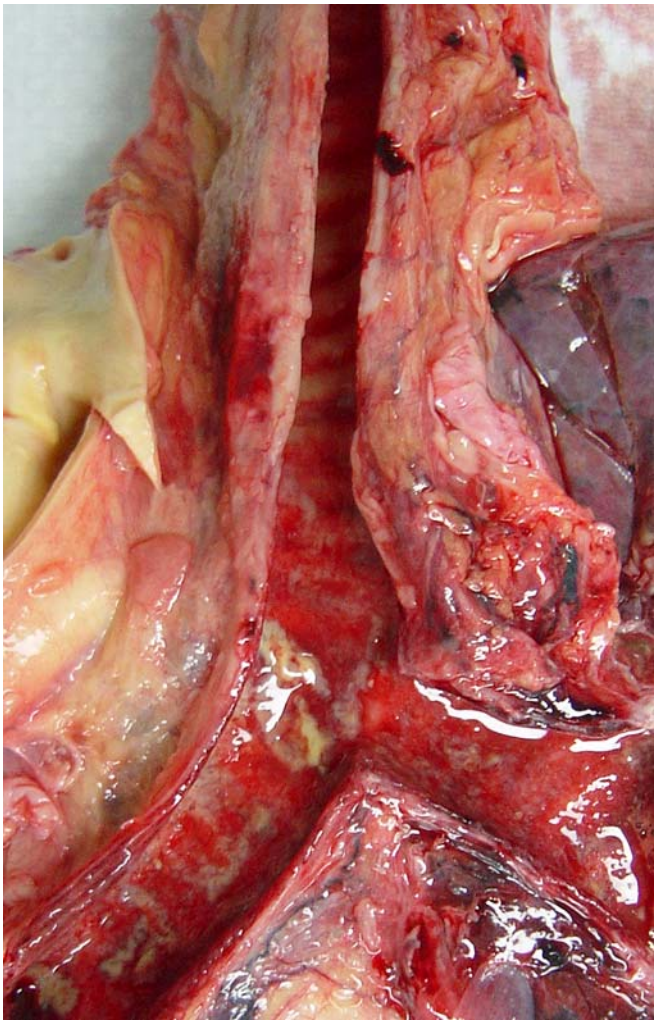

Fig. 1 Macroscopic appearance of hemorrhagic tracheobronchitis with the mucosal surfaces partly covered by purulent membranes

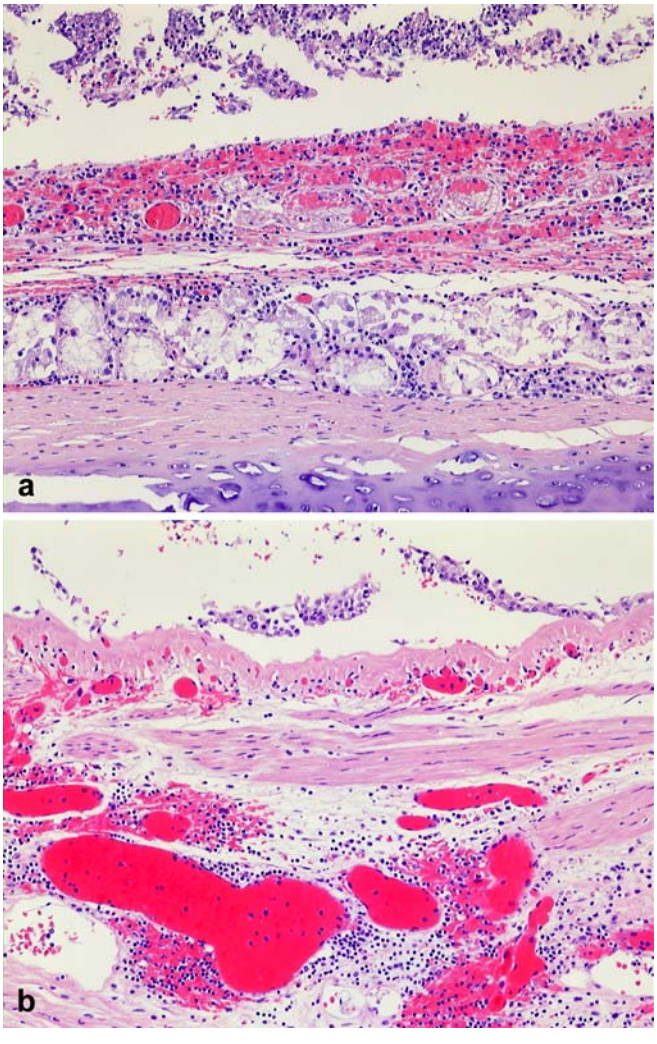

Fig. 2 Mucosal ulceration and necrosis of trachea **a** and bronchus **b** with dense lympho-monocytic infiltration of all layers accompanied by vascular congestion (hematoxylin and eosin, original magnification ×50)

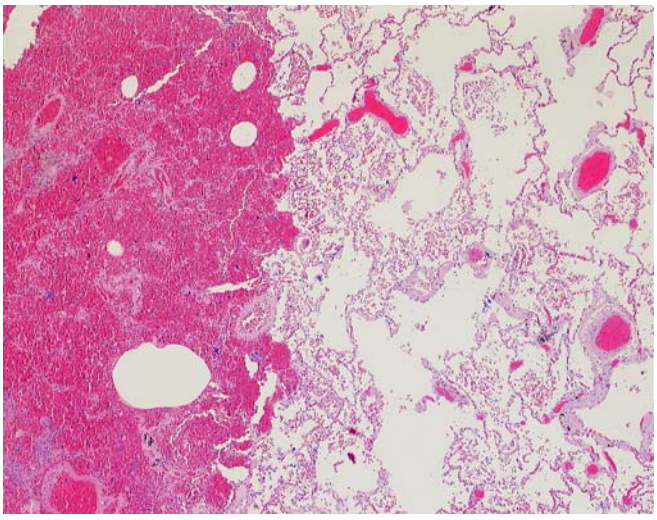

Fig. 3 Hemorrhagic pneumonia strictly limited to lobular outlines. Congestion of pulmonary vessels and a marked perivascular edema is seen in both inflamed and pathologically unchanged pulmonary tissue (hematoxylin and eosin, original magnification ×10)

Polymerase chain reaction (PCR) for detection of influenza virus was done from fresh tissue samples taken from the bronchi and lungs that were obtained during autopsy. After reverse transcription with random nonamers PCR was performed with primers Flu-AM151 (5'-CATGGAATGGCTAAAGACAAGACC-3') and Flu-AM397 (5'-AAGTGCACCAGCAGAAT AACTGAG-3') targeting the M gene of the influenza A virus which encodes for the viral matrix protein. PCR was carried out using standard conditions for amplification. Influenza A virus was detected in the bronchi and lung samples (Fig. 4). PCR assays for *Mycoplasma pneumoniae*, *Chlamydia pneumoniae, Ureaplasma urealyticum*, human metapneumovirus, adenovirus, influenza B virus, parainfluenza virus 1 and 3, and respiratory syncytial virus yielded negative results. Sequencing of the influenza A amplicons utilizing primers Flu-AM151 and Flu-AM397 revealed influenza A virus type H3N2 in a BLAST analysis (NCBI database, http://www.ncbi.nlm.nih.gov/BLAST).

Blood alcohol concentration was negative as determined in a femoral venous blood sample.

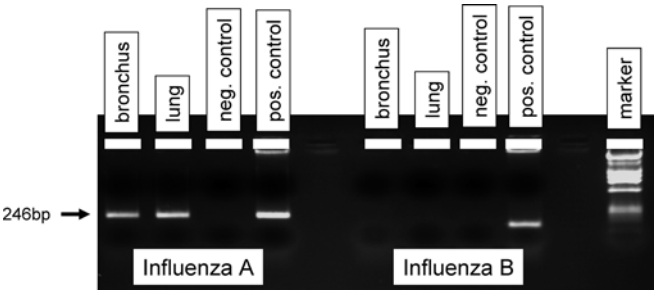

Fig. 4 Detection of influenza A virus in bronchus and lung samples by PCR. The primers target the viral M gene leading to an amplicon of 246 bp. Influenza B virus could not be detected in a separate PCR assay

Death was attributed to acute respiratory failure due to necrotizing tracheobronchitis and hemorrhagic pneumonia caused by influenza A with *Staphylococcus aureus* superinfection and the manner of death was stated as natural.

According to the deceased's family physician who was contacted after knowledge of the microbiological results, the woman's previous medical history had been unremarkable. She had not received any influenza vaccinations in previous years.

Discussion

We describe the fatal case of influenza A infection with *Staphylococcus aureus* superinfection in a previously healthy 49-year-old woman. Autopsy revealed severe necrotizing tracheobronchitis and hemorrhagic pneumonia. Influenza A infection was proven by PCR and bacterial superinfection with *S. aureus* was shown by culture from tracheal, bronchial and pulmonary swabs obtained during autopsy.

Fatal influenza infection can be caused by influenza pneumonia alone, by respiratory complications caused by bacterial superinfection or by extrapulmonary influenza-associated manifestations such as myocarditis or encephalopathy [6, 8, 9]. In the present case, extrapulmonary infectious alterations were ruled out histologically.

The knowledge of the pathology of influenza pneumonia is based mainly on observations derived from autopsy specimens obtained during the 1957–1958 epidemic [11, 12, 13, 14] and from rare non-epidemic fatal cases [6, 7, 15]. The histopathological features of influenza pneumonia are remarkably consistent, regardless of the type of strain or virus or the location of the infection, and similar histopathological changes have been observed in lung biopsy specimens [15]. However, it has been shown by different investigators that it is difficult to distinguish pulmonary changes due to influenza pneumonia from secondary staphyloccocal infection histologically, if lung biopsy specimens are obtained in acute stages of the disease [15, 16].

Influenza virus infection rates are highest in children [17]. By using the nested polymerase chain reaction (PCR) for the detection of different RNA viruses in postmortem lung tissue samples from 114 infant deaths, Bajanowski et al. [18] found a positive virus detection of influenza B in 5 cases. Complication rates of influenza infection are highest in individuals with chronic pulmonary and cardiovascular diseases, immunosuppressed patients, diabetics or individuals with other severe metabolic illnesses, subjects older than 65 years old [19, 20, 21, 22] and, as recently described, superinfection with a highly pathogenic *Staphyloccoccus aureus* strain carrying the gene for Panton-Valentine leukocidine [10]. In this case none of the aforementioned conditions or other predisposing factors could be established. Therefore, based on the circumstances observed in the present fatality and in the light of clinical data reported on influenza epidemic fatalities that

also affected younger, immunocompetent adults [11, 12, 13, 14], one is tempted to speculate that not only elderly individuals or persons with high-risk medical conditions may substantially benefit from vaccination, which has a high effectiveness (90%) in healthy individuals [23]. Vaccination has been shown to reduce the mortality of influenza even with bacterial superinfection by 70% [24]. Unfortunately, in Germany for example the immunization rates even in risk groups range from only 13–63% [25].

Apart from the noteworthy observation that influenza A infection complicated by bacterial superinfection can run a fatal course even in previously healthy, younger adults, this case most significantly demonstrates the need for an interdisciplinary approach towards an organism-specific diagnosis of potentially infection-related deaths undergoing autopsy. Most remarkably, the highly instable RNA of influenza A virus could be detected in pulmonary autopsy specimens obtained 30 h postmortem. Therefore, with improved diagnostic possibilities such as PCR and sequencing, forensic pathologists can, in close association with the field of microbiology, make a significant contribution to one of the central public health issues, namely the detection of highly infectious agents, which must be notified to the authorities. This will increase particularly the knowledge about the influence of these agents on sudden, unexpected deaths in outpatients.

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