Chapter 2

Pathology of Influenza

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Additional information is available at the end of the chapter

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Abstract

The chapter is based upon the own experience of scientific school the author belongs in comparison with the published data. Special attention is paid to three types of cellular changes related to viral replication, immediate causes of death, extrapulmonary lesions, and peculiarities of lesions in experimental mice model. Different courses of the disease and morphologic appearance during different epidemics are analyzed as well.

Keywords: influenza, histopathology, immediate death causes

1. Introduction

Influenza A and influenza B can present along a broad spectrum of disease, ranging from subclinical to severe generalized infection leading to numerous lethal outcomes causing pandemia and epidemics or developing as sporadic cases [1]. Particular attention of clinicians, physicians, virologists, and molecular biologists, as well as public media, is devoted to the lesions of respiratory organs during the epidemics with most severe course of the disease. Only in clinical practice such cases usually are officially registered as influenza. Pathological studies are much less in number and usually devoted only to changes in the trachea and lungs in single cases or small series of autopsy observations. In majority of manuals, pathology in influenza is characterized as vascular disorders or viral pneumonia [2, 3]. No typical changes related to virus replication are discussed. The data related to histopathological changes in extrapulmonary lesions are very scarce in spite of widely accepted fact that influenza negatively influences upon the course of myocardial infarction and other forms of ischemic heart disease. It has been considered for a long time that the most severe illness with possible lethal outcomes usually develops in infants and elderly persons due to decreased immune reactions. In 2009–2011 during the epidemics caused by H1N1 virus (so-called swine strain) all over the world, the most of lethal

outcomes occurred in middle-age patients with the signs of metabolic syndrome, but this fact has not been explained in the literature [4–9]. The fact that influenza has more severe course in pregnant women has been described by many authors [10], but its pathogenesis remains unclear. In the literature, one cannot find special analysis of immediate death causes related to different periods of time, virus strains, and age of the deceased.

In Saint Petersburg/Leningrad (Russia, former Soviet Union), pathology of influenza has been studied by Zinserling (Tsinzerling) (1923–1995), his collaborators, and pupils since the end of 60th. The most of numerous publications of that time were in Russia, partly summarized in monographs and manuals [11], but he succeeded to make several contributions in the world literature as well [12, 13]. The most significant data received at that time was demonstration of typical cell changes, called by him "influenza cells." Cytoplasm enlarged being at early stages of the disease basophilic, later on parallel with virus disappearance pale stained and showing signs of degeneration. Intracytoplasmic fuchsinophilic inclusions were considered as very typical, although not specific, for influenza, representing the necrotic foci surrounded by the membrane. Appearing as small dots in the perinuclear zones, they grew in size and were transferred at the periphery being expelled afterward. Such transformations were observed in ciliated epithelial cells of the trachea and bronchi, alveolocytes, and lung macrophages. As exception such inclusions were noted also in smears from meninges and placenta. In infants and small children basing upon clinical, virological data and immunofluorescent microscopy, generalized infection was proven in many cases with development of brain, liver, kidney, intestine, and adrenal lesions partly with appearance of "influenza cells." In adults the majority of lethal cases were explained by bacterial, at that time usually staphylococcal, superinfection leading to destructive pneumonias. Mucous layer in the larynx, trachea and bronchi was swollen with mixed infiltration and desquamation of epithelium. Viral antigen could also be detected in capillary endothelium. Described changes were found in all cases of influenza due to viruses H3N2 and B, regardless of its clinical manifestation. In cases with expressed clinical symptoms, lung edemas and plethora of vessels with hemorrhagic foci were observed. Neutrophilic infiltration was considered as a hallmark of bacterial superinfection. Indeed, in majority of cases of focal pneumonias, viral-bacterial associations have been found. Bacterial pneumonia can be both community and hospital acquired.

Later on the progress of clinical and preventive medicine resulted in critical decrease of lethal outcomes due to influenza. Situation changed with appearance of the new "swine" strain of virus. Our new experience was partly summarized in Russian [14, 15] and international press [16].

Pathomorphological features and disease severity depend on patient general state and susceptibility, as well as the virus type in question. The so-called H1N1 avian influenza virus is considered to be the most dangerous virus, causing generalized infection with more than 50% lethality. Seasonal H3N2 and H1N1 influenza viruses that have been circulating in recent years tend to cause primarily localized respiratory infection, although extrapulmonary lesions may occur in severe cases. New H1N1 influenza virus also causes mainly localized infection, but in most autopsies, signs of generalization can be found. Also, it should be noted that bacterial coinfections are relatively rare.

Diagnosis is based upon the epidemiological, clinical, and virological data. At the autopsy enlarged slightly firmed reddish lungs were very typical (**Figure 1**), while in histological examination, the changes of the infected cells becoming enlarged intensively stained cytoplasm were very informative. In other respiratory infections, either cytoproliferative changes (respiratory-syncytial and parainfluenza) or intranuclear basophilic inclusion (adenoviral and respiratory herpes) is notified.

Our experience based upon about thousand observations in the period 1977–2017 allows us to make several not widely known statements:

1. Clinical course of influenza may differ, and sometimes, usually in intraepidemic periods, the disease may manifest with moderate symptoms usual for all acute respiratory infections of different viral etiologies (rhinitis, cough, etc.).

2. Our data allow to confirm the existence of previously described in experiment chronic forms of influenza without distinct clinical symptoms, being able to be activated in unfavorable for the patient situation, superinfection by other pathogens first of all. Persistence of viral antigens in lung macrophages without signs of inflammation has been proven in our study with the help of immunohistochemistry (**Figure 2**). One can submit that this phenomenon has to be studied specially and can be considered in relation to survival of "not actual" viral strains. Once, we had the opportunity to detect such strain in plexus choroideus of 4.5 month girl.

3. It is widely accepted and properly investigated that all types and antigenic variants of influenza virus have tropism to ciliated epithelial cells of the trachea and bronchi of different calibers. In single publications it has been demonstrated that alveolocytes, lung macrophages, and endothelial cells can be considered as targets for virus as well (**Figure 3**). In accordance with our experience, virus-exposed cells are submitted to typical transformation which can differ due to properties of virus strain. During the infection caused by virus with short replicative period (H3N2 as example), one can observe during the first 3 days of the infection of the cells with the enlarged slightly basophilic cytoplasm ("influenza cells" of Zinserling) (**Figure 4**). Such changes were explained by active viral replication. Later on (5th–7th day

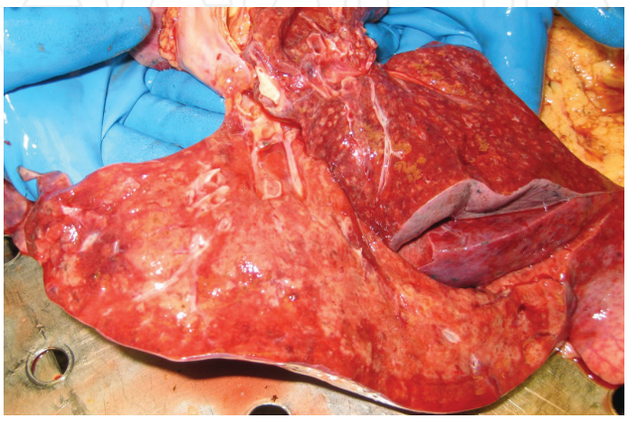

Figure 1. Macroscopic view of the lung of a deceased patient from influenza A H1N1 California pdm "swine".

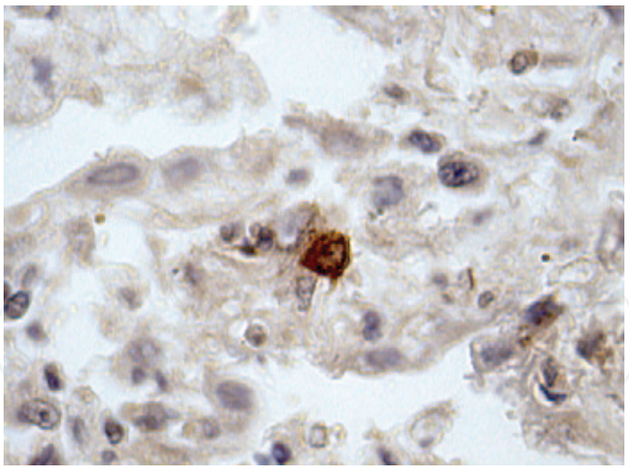

Figure 2. Antigen of influenza A virus nucleoprotein in cytoplasm of interstitial alveolar macrophages without any inflammatory changes in a patient without signs of respiratory infection (IHC, ×400).

of the disease), the cells are desquamated and undergo necrotic changes, which are hallmarked by cytoplasm becoming pale with indistinct cell borders. In the infection due to strains with prolonged virus replication in lungs (H1N1 California first of all), one can assume that virus-cell interactions undergo important modifications and the infected cells instead of dying express proliferative changes. In our experience they can become binucleated (**Figure 5**), considerably enlarged (**Figure 6**), multinucleated (**Figure 7**), or even undergo squamous cell metaplasia (**Figure 8**). Comparison between two types of cell changes due to influenza virus is presented in **Table 1**. From **Table 1** it is evident that recently described influenza cells of the two types typical for influenza H1N1 California are larger ($p \leq 0,05$) than influenza cells described and studied by Zinserling and are distinguished from them also by larger nucleus-cytoplasm index ($p \leq 0,05$). It is evident that the fine mechanisms of such only recently described phenomenon need further study.

4. In many lethal cases due to influenza, we deal with mixed infection. The most evident variant of its development is bacterial superinfection in the course of influenza leading to

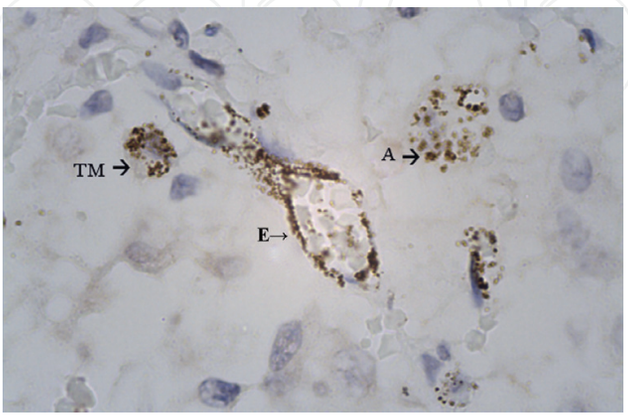

Figure 3. Immunohistochemical detection of influenza virus HA in lungs in lethal H1N1 influenza (×1000): TM, alveolar macrophage; Э, endothelium; A, alveolocyte.

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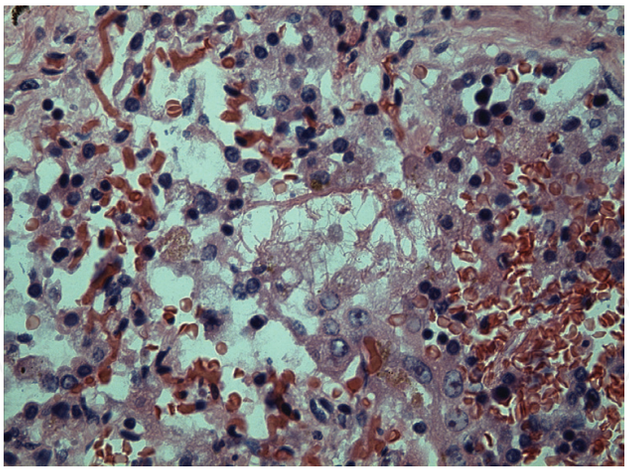

Figure 4. Lungs in lethal H1N1 influenza: the first type of virus-affected ("influenza") cells with prominent hyperemia and dystelectasis (H&E, ×400).

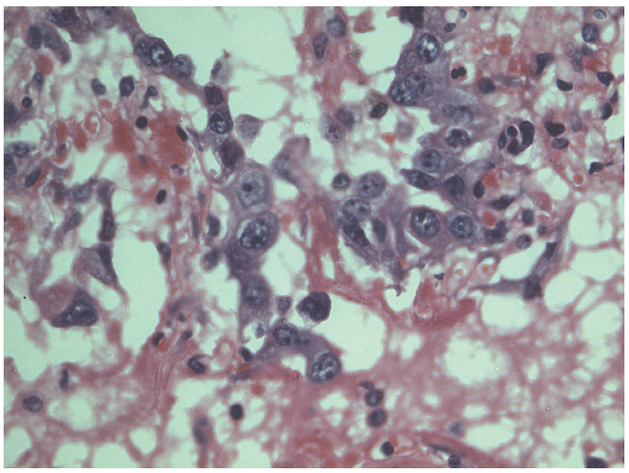

Figure 5. Lungs in lethal H1N1 influenza: the second type of virus-affected ("influenza") cells (H&E, ×600).

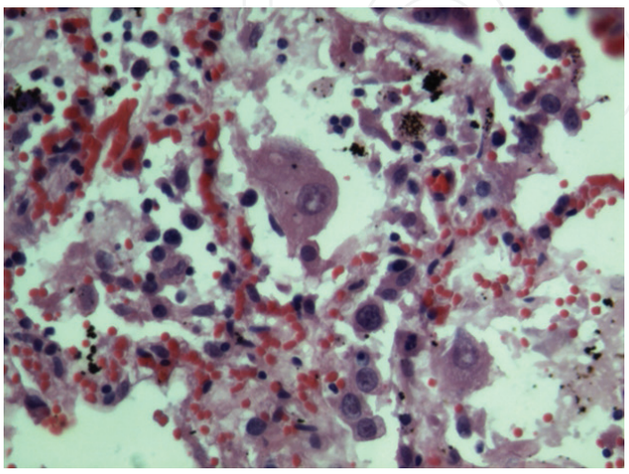

Figure 6. Enlarged alveolar macrophages (H&E, ×600).

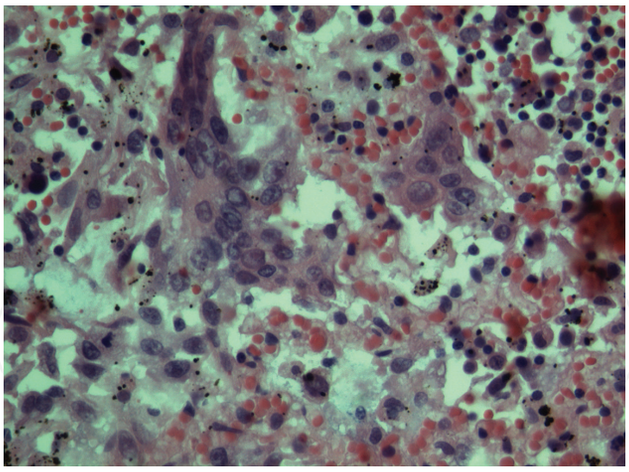

Figure 7. Giant multinuclear cells (H&E, ×600).

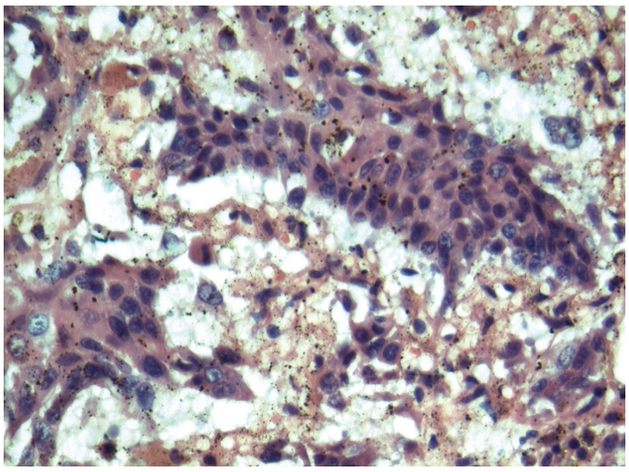

Figure 8. Squamous cell metaplasia (H&E, ×600).

	Influenza cells (first type)	Influenza cells (second type)
Square (relative unit)	14,273.6 ± 1099.8	19,715.3 ± 1436.4
Nucleus-cytoplasm index	0.274 ± 0.014	0.347 ± 0.016

Table 1. Square and nucleus-cytoplasm index in influenza cells of the first and second type ($n = 50$; $p < 0.05$).

defect of defense mechanisms of respiratory tract due to desquamation of ciliated cells. Later on viral-bacterial pneumonia develops playing an important role in clinical manifestations and tanatogenesis in lethal cases. Bacterial pneumonias are focal, usually confluent, frequently with signs of tissue destruction. The etiology of such bacterial superinfection can differ, in certain periods of time with prevalence of staphylococci, but in majority of cases stays undetermined either clinically or at the autopsy. Interestingly that during the lethal outcomes in 2009–2011 with the leading role in pathogenesis of diffuse alveolar damage (DAD), the expression of neutrophilic infiltration usually explained by bacterial component

was rather modest. Certainly, this fact can partly be explained as a result of the efficacy of antibiotic treatment, but the same tendency was noted also in the patient without it.

Another case demonstrates practically not discussed in the literature variant of development of mixed infection is activation due to influenza virus influence of another preexisting, but not manifestating infection. One of our observations allows to suspect such possibility with high probability.

Case report of lethal influenza A/H1N1/California

One of the studied cases is of particular interest. We succeeded to provide more detailed investigation using immunohistochemical detection of *Chlamydia* spp., *Adenovirus*, influenza H1N1, *Herpes simplex* type 1 and type 2 (HSV1/HSV2), cytomegalovirus (CMV), Epstein–Barr virus (EBV), RSV, enterovirus, parvovirus, *Mycoplasma pneumoniae*, as well as electron microscopy. The latter was performed as follows: samples were fixated in glutaraldehyde 4 hours after death and then treated with osmium oxide, and uranyl acetate was used for contrast enhancement. Dehydration and embedding were performed using conventional techniques. Sections were analyzed on electron microscope JEM-100S.

A 31-year-old male patient IV with obesity got ill acutely, with fever up to 39°C. Three days later he was hospitalized to the intensive care unit with severe respiratory insufficiency. Intubation and, later on, tracheostomy were performed. The clinical diagnosis of "swine influenza" was supported by RT-PCR and serology (increase of the antibody level from 0 to 640). In spite of intensive treatment including antibiotics and antiviral drugs, the patient died on the 35th day of the illness.

Macroscopic changes. At the autopsy remarkable changes were found only in the lungs: necrotic posttracheostomic tracheitis, large abscesses in the lower lobes of the lungs in the stage of organization, and bilateral fibrinous pleuritis. The rest of the lung tissue was dark red and firm.

Microscopic changes. During the histological examination in the lungs, changes typical for rather old abscesses and late stages of respiratory distress syndrome were noted (**Figure 9**). The changes that we consider to be typical for influenza (virus-induced transformation of epithelial cells) were expressed only in the moderate number of the cells. We notified numerous intra-alveolar macrophages, partly with vacuolated cytoplasm (**Figure 10**), and PAS-positive inclusions. Some cells had slightly enlarged hyperchromic nuclei. Similar changes were noted in other organs as well.

Postmortem laboratory investigation. Postmortem RT-PCR of lung and spleen specimens for influenza A/H1N1sw was negative. During the bacteriological investigation of lung specimens, cultures of *E. coli*, nonpathogenic *Corynebacterium*, *Enterococcus*, *S. viridans*, and *S. epidermidis* were isolated.

Immunohistochemical investigation. In lungs strong positive reactions with serum against *Chlamydia* spp. (**Figure 11**), moderate against *Adenovirus* (**Figure 12**), and weak against influenza H1N1 were obtained. The reactions with sera against HSV1/2, CMV, EBV, RS, enterovirus, and parvoviruses, as well as *Mycoplasma pneumoniae*, were negative.

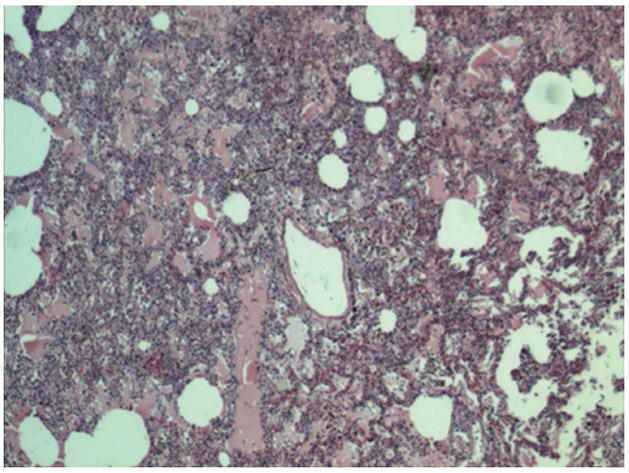

Figure 9. Lungs of a patient IV deceased from H1N1 influenza at low-power magnification, signs of moderate DAD (H&E, ×100).

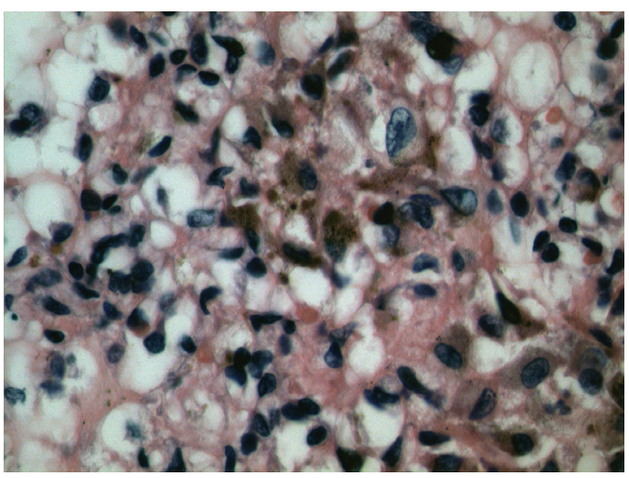

Figure 10. Lungs of the same patient, prevalence of macrophages in the exudate (H&E, ×640).

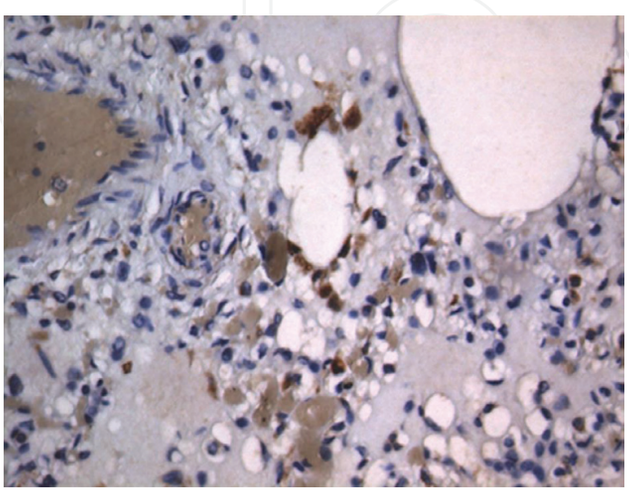

Figure 11. Lungs of the same patient, IHC with serum against *Chlamydia* spp. (×400).

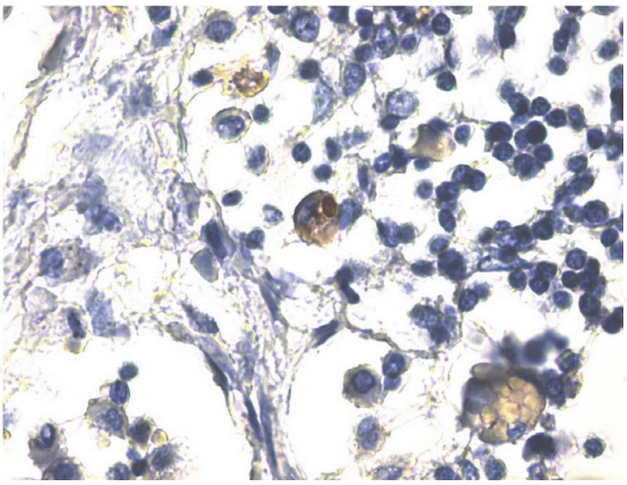

Figure 12. Lung lymph node of the same patient, IHC with serum against adenovirus (×640).

Electron microscopy. During the electron microscopic investigation of lungs, we succeeded to evaluate numerous elementary and reticular bodies of *Chlamydia* (**Figure 13**), in the brain only reticular bodies, predominantly in the cytoplasm of endothelial cells. In their nuclei several PML nuclear bodies (small intranucleolar inclusions containing promyelocytic leukemia protein) were found (**Figure 14**).

Conclusion. Death of a young previously healthy man occurred on the 35th day of illness clinically regarded as influenza with bacterial superinfection. The results of postmortem morphological and laboratory investigation proved that clinically diagnosed infections were expressed rather weakly but provoked the activation and severe course of respiratory chlamydiosis (with probable generalization) and adenoviral infection.

5. Practically, all authors from all over the world describing the lethal outcomes due to epidemic strain of influenza A virus underline the development of DAD syndrome with respiratory insufficiency resistant to treatment. During the postmortem morphological investigation macroscopically, firm reddish lungs vaguely resembling red congestion seen in

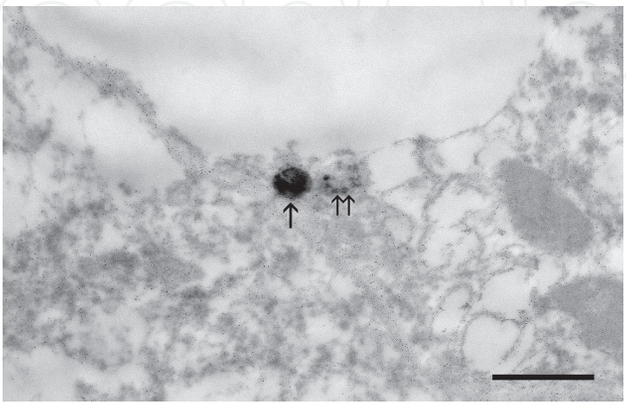

Figure 13. Lungs of the same patient, elementary (double arrow) and reticular (single arrow) bodies of *Chlamydia* detected by electron microscopy (scale 1 μm).

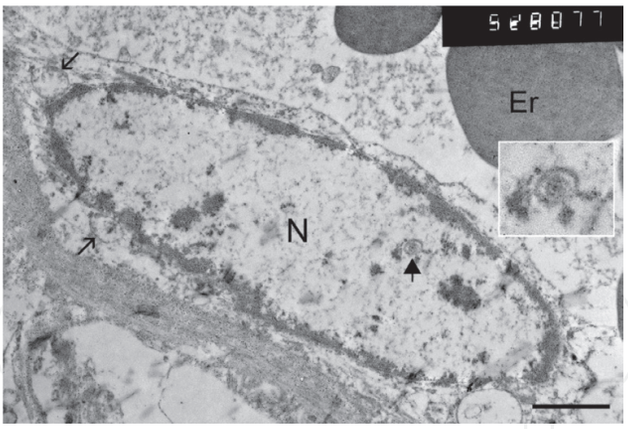

Figure 14. Brain of the same patient, reticular bodies in the cytoplasm and PML inclusion (insertion on the right) in endothelial cell detected by electron microscopy. N, nucleus; Er, erythrocyte (scale 2 μm).

lobar pneumonia are noted. But microscopic picture is quite different: lungs are plethoric; lumen alveoli contain serous exudate (but practically no neutrophils), occasionally erythrocytes; and the most typical and important were hyaline membranes (**Figure 15**). Microthrombosis of arterioles and venules was noted as well (**Figure 16**). Such changes were not typical for lethal outcomes due to former virus types. We can assume that such changes could be explained by cytokine overproduction by damaged macrophages, but such hypothesis needs to be proven by proper methods.

6. Extrapulmonary manifestations with involvement of the brain, meninges, heart (**Figure 17**), vessels, kidneys, liver, and intestine in severe influenza occur rather regularly. Partly, they can be explained by vascular disorders due to not fully clarified "toxic influences," but tropism of certain influenza strains to the brain, intestine, endothelium, and placenta has been either proven or at least suspected. There is also evidence of intrauterine influenza, although usually without severe clinical manifestations. The role of influenza virus in development of malformations has been discussed for a long time but still remains doubtful. The question which

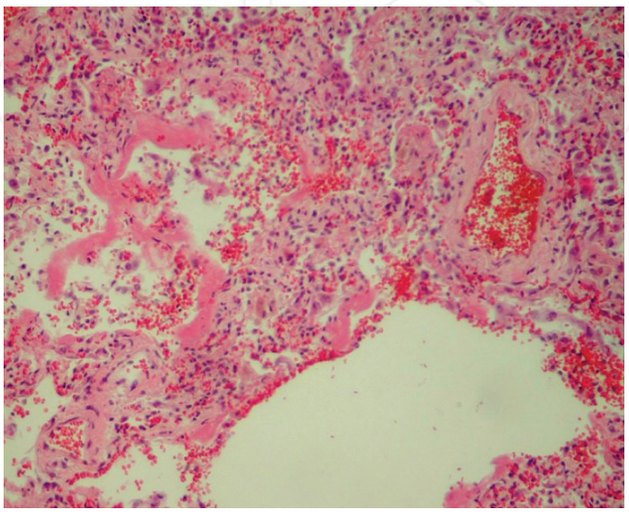

Figure 15. Hyaline membranes in the lung of a patient deceased from influenza a H1N1 swine (H&E, ×600).

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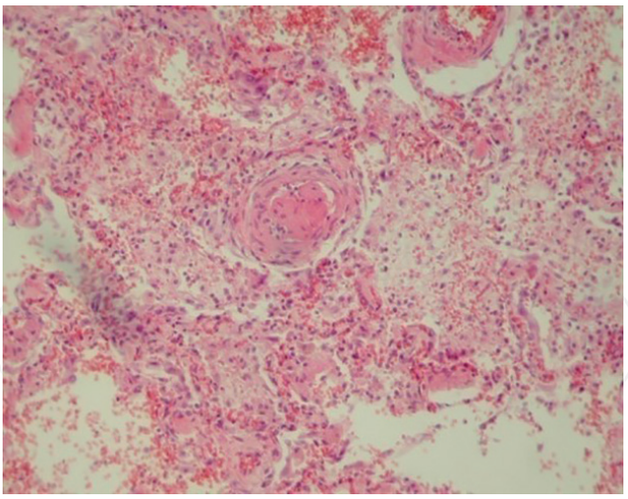

Figure 16. Microthrombosis of the arteriole in the lung of a patient deceased from influenza a H1N1 swine (H&E, ×400).

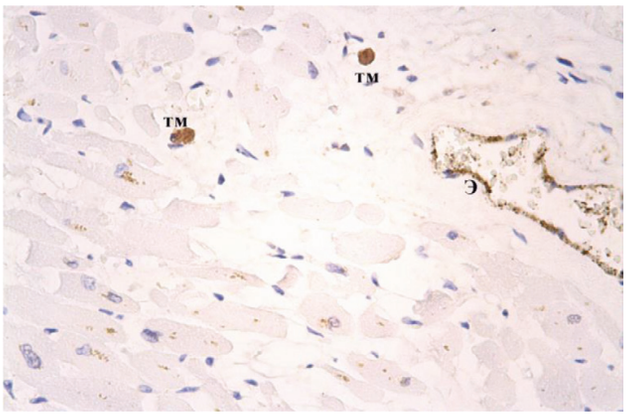

Figure 17. Nuclear protein of influenza a virus in the heart (IHC ×400).

needs further studies is the fact that the antigen of influenza virus is detected outside the respiratory system with the help of IHC or immunofluorescence more frequently than by virus cultivation. The hypothesis of probable appearance of incomplete viral particles or tis-mutants needs to be investigated on clinical material.

7. Immediate death causes in influenza may be quite different. Basing upon our long-term experience, we can distinguish (1) severe respiratory insufficiency due to respiratory distress syndrome, (2) generalized viral infection, (3) secondary bacterial pneumonia, and (4) aggravation of severe somatic diseases. Many aspects of pathogenesis need complex study. It is obvious that such division has extreme clinical importance; unfortunately, we never found similar analysis in the literature.

In experiment the following groups were studied: (1) mice intranasal challenged with influenza viruses A/California/07/09 (H1N1) and A/WSN (H1N1) in saline solution in doses 1 and 10 LD50, and (2) control mice got only saline or were considered as negative control. Virus titer was determined by standard methods.

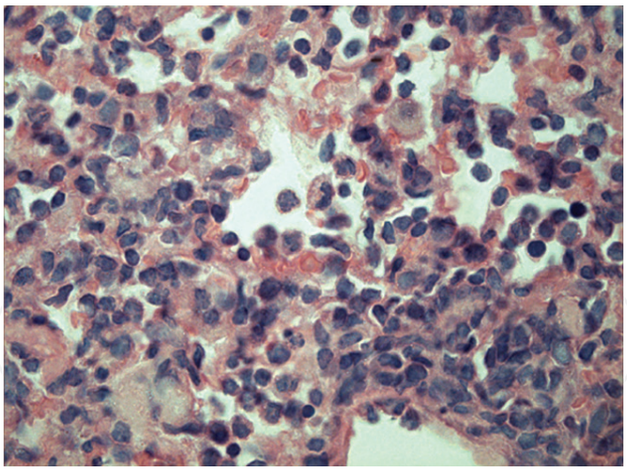

Figure 18. Virus-induced changes in the lung of a mouse on 3D after intranasal challenge with influenza a/California/07/09 (H1N1) (H&E, ×600).

Replication of influenza viruses of both types was maximal on the third day after challenge (California 6.20 ± 0.18; WSN 6.10 ± 0.26) with its gradual decrease on the 7th and 14th day. One has to note that on the 14th day the medium index of replication influenza California virus (1.30 ± 0.30) was more than twice as high as those of A/WSN 0.60 ± 0.22).

The cells of bronchial epithelium, alveolocytes, alveolar macrophages, and endotheliocytes contained viral antigen and underwent changes similar to those described on human autopsy material but expressed moderately (**Figure 18**). Lesions comparable with "diffuse alveolar damage" in men were absent.

Thus, we can resume that the disease caused by different strains of influenza A virus has substantial peculiarities. Histopathological changes are rather typical and allow at least to suspect the etiology of infection, which has to be confirmed by virological methods, RT-PCR nowadays first of all. Lethal outcomes may be related to (1) severe respiratory insufficiency due to respiratory distress syndrome, (2) generalized viral infection, (3) secondary bacterial pneumonia, and (4) aggravation of somatic diseases, ischemic heart disease first of all. Many questions of influenza pathogenesis need to be clarified in complex studies on clinical and experimental material including morphological methods. Among the most important studies which have to be provided, we can mention (1) the exact virus-cell interactions in the target cells and mechanism of cytoproliferative changes; (2) interactions of influenza with other viruses, bacteria, mycoplasma and fungi; (3) the nature of extrapulmonary lesions; and (4) the difference in histopathological picture due to the same virus type in different species.

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References

[1] Thangavel RR, Bouvier NM. Animal models for influenza virus pathogenesis, transmission, and immunology. Journal of Immunological Methods. Aug 2014;**Apr 4**:60-79. DOI: 10.1016/j.jim.2014.03.023

[2] Kradin RL, editor. Diagnostic Pathology of Infectious Disease 1st ed. Philadelfia: Saunders Elsevier; 2010. ISBN: 978-1-4160-3429-2

[3] Milner DA, editor. Diagnostic pathology. In: Infectious Diseases. 1st ed. Philadelfia: Elsevier; 2015. ISBN: 978-0-323-37677-8

[4] Gill JR, Sheng Z-M, Ely SF, et al. Pulmonary pathologic findings of fatal 2009 pandemic influenza A/H1N1 viral infections. Archives of Pathology & Laboratory Medicine. 2010; **134**:235-243

[5] Mauad T, Hajjar LA, Callegari GD, et al. Lung pathology in fatal novel human influenza A (H1N1) infection. American Journal of Respiratory and Critical Care Medicine. Jan 1, 2010;**181**(1):72-79

[6] Nakajima N, Hata S, Sato Y, et al. The first autopsy case of pandemic influenza (A/H1N1pdm) virus infection in Japan: Detection of a high copy number of the virus in type II alveolar epithelial cells by pathological and virological examination. Japanese Journal of Infectious Diseases. 2010;**63**:67-71

[7] Bhatnagar J, Jones T, Blau DM, et al. Localization of pandemic 2009 H1N1 influenza A virus RNA in lung and lymph nodes of fatal influenza cases by situ hybridization: New insights on virus replication and pathogenesis. Journal of Clinical Virology. 2013;**56**(3):232-237. DOI: 10.1016/j.jcv.11.014

[8] Capelozzi VL, Parra ER, Ximenes M, et al. Pathological and ultrastructural analysis of surgical lung biopsies in patients with swine-origin influenza type A/H1N1 and acute respiratory failure. Clinics. 2010;**65**(12):1229-1237

[9] Merzt D, Geraci J, Wincup J, et al. Pregnancy as a risk factor for severe outcomes from influenza virus infection: A systemic review and meta-analysis of observational studies. Vaccine. 2016;**35**(4):521-528. DOI: 10.1016/j.vaccine.2016.12.012

[10] Zinserling (Tsinzerling) VA. Etiology and Pathologic Anatomy of Acute Respiratory Infections (in Russian). Leningrad: Meditsina; 1977

[11] Zinserling VA. Peculiarities of lesions in viral and mycoplasma infections of respiratory tract. Virchows Archiv. 1972;**356**:259-273

[12] Zinserling VA, Aksenov OA, Melnikova VF, Zinserling VA. Extrapulmonary lesions in influenza. Tohoku Journal of Experimental Medicine. 1983;**140**:259-272

[13] Zinserling VA, Vorobjev SL, Zarubajev VV, et al. Pathogenic aspects of influenza during the epidemics caused by virus H1N1v in 2009-2010 on autopsies. Arkhiv Patologii (Moskwa). 2011;**73**(6):21-25 (in Russian)

[14] Yakovlev AA, Zinserling VA, Esaulenko EV. Lethal outcomes in influenza: Clinic-pathological approach to immediate death causes (in Russian). Journal of Infectologii. 2017;**9**(4):53-59. DOI: 10.22625/2072-6732-2017-9-4-53-58

[15] Zinserling VA, Dedov VA Influenza. In: Hofman P, editor. Infectious Disease and Parasites. Berlin: Springer; 2016. DOI: 10.1007/978-3-319-300009-2

[16] Zinserling VA. Immediate death causes in influenza. Virchows Archiv. 2017;**471**(S.1):s100