資料４－２

インフルエンザが輸血により伝播する可能性についての文献調査

関連各種論文等（要約）一覧表

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資料番号			概要
①	N Engl J Med. 1963. 31; 269:964-6	Human Influenza Infection with Proved Viremia, Report of a Case	発症後4日の血液からインフルエンザウイルス（A型Type2)が分離された症例報告。
②	Trans Assoc Phys. 1966: 79: 376-377	Viremia in Asian Influenza	原文取り寄せ中
③	British Medical Journal 1969:4, 208-209	Proved viraemia in Asian influenza (Hong Kong variant) during incubation period	21例のインフルエンザ様症状の患者うち、12例の咽頭ぬぐい液からウイルスを検出。その他潜伏期間中にあった1例より咽頭ぬぐい液及び血液よりウイルスを検出。
④	Can Med Assoc J. 1976 September 4; 115(5): 435–437	Postsplenectomy sepsis due to influenza viremia and pneumococcemia	原文取り寄せ中
⑤	J Hyg Epidemiol Microbiol Immunol. 1979;23(1):35-41	Investigation of the incidence of influenza A viraemia caused by virus strains circulating among children in 1968 – 1977	原文取り寄せ中
⑥	Clin Infect Dis. 1997 Apr;24(4):736-737	Use of the polymerase chain reaction for demonstration of influenza virus dissemination in children	インフルエンザ患者14名の有症状時の血液を調べたところ、いずれからもウイルスは検出されなかった。
⑦	Journal of Medical Virology 58:420–425 (1999)	Detection of Influenza Virus RNA by Reverse Transcription-PCR and Proinflammatory Cytokines in Influenza-Virus-Associated Encephalopathy	インフルエンザ脳症の小児患者でのウイルス同定調査結果。咽頭スワブで100%(9/9)、血漿で0%(0/11)、ＰＭＢＣ（末梢血単核球）で11%（1/9)、赤血球で0%（0／9）、脳脊髄液で9%（1／11）であった。インフルエンザ脳症を起こしていないコントロール群では、咽頭スワブで100%(29/29)であったが、血漿、末梢血単核球、赤血球のいずれからも同定されなかった（0/29)。
⑧	WHO, 19 May 2006	Maintaining a Safe and Adequate Blood Supply in the Event of Pandemic Influenza: Guidelines for National Blood Transfusion Services	インフルエンザへの血液を介しての感染のリスクは極めて低い。これまで、輸血を介してインフルエンザに感染したという報告はなく、呼吸器疾患ウイルスが輸血を介して感染することは、ウイルス量が極端に多い場合を除き、起こりそうにない。重要なことは、（パンデミック下では）血液を通じて感染するリスクは、呼吸器を通じて感染するリスクより、よほど低いことである。
⑨	Transfusion, 47, 1071-1079 (2007)	Planning for pandemic influenza: effect of a pandemic on the supply and demand for blood products in the United States	鳥インフルエンザウイルスのパンデミック対応。1918年のパンデミックインフルエンザであるいわゆる「スペインかぜ」についての検証を行っている。パンデミックにより、血液製剤の採血、製造、輸送に大きな影響が起こりうる。血液サービスで働く従業者も大きく減少する可能性がある。インフルエンザウイルスが輸血によって伝播したという報告は無い。また、一般にインフルエンザを発症していても血液からウイルスが検出されることは無い。しかし、高病原性鳥インフルエンザH5N1のベトナム株やインドネシア株では感染した子供の血清中や血漿中にウイルスが存在するという報告がある。しかし、インフルエンザウイルスのウインドウ期はきわめて短いと想定されることから、歴史的にインフルエンザウイルスが輸血により感染する可能性は低いとされてきているが、H5N1の場合には伝播の懸念が否定できない。

資料番号			概要
⑩	Transfusion, 47, 1080-1088 (2007)	Influenza viremia and the potential for blood-borne transfusion	鳥インフルエンザウイルスのパンデミックが起こった場合を想定し、輸血によるウイルス伝播の可能性について考察した。これまで輸血によるインフルエンザウイルスの伝播について報告された事例は無い。インフルエンザウイルス血症は極めてまれにしか起きないこと、及び無症候の献血者からしか採血されないことを考えると、血液によってウイルスが伝播する可能性はきわめて低いと想定される。仮に輸血によりインフルエンザウイルスの伝播が起こるとすると、輸血を受けた免疫抑制状態の患者では重症化や致死率が上昇する可能性はある。ウイルス血症に関するデータは殆ど無く、1960-1970年代の古いデータである。殆どのデータが発症後にサンプリングされた検体でのデータである。インフルエンザ脳症を起こしている患者の血液や脳髄液にはウイルスが検出されることはまれなのに、インフルエンザ脳症患者では全身にウイルスが広がることが示唆されている。このことは、脳神経症状の発症には脳髄液でのウイルスの存在は必要がないこと、換言すれば、ウイルス血症や脳脊髄液でのウイルスの出現の前に、インフルエンザ脳症が発症しうることを意味しているかもしれない。
⑪	Vaccine, 26, D59-D66 (2008)	Pathology of human influenza revisited	H5N1は肺や気管支上皮に感染しやすく、そのために感染部位から拡散しやすい性質を持つ。季節性インフルエンザと異なり、H5N1はウイルス血症及び呼吸器系外へ感染が広がる可能性が高い。H5N1がウイルス血症を起こす可能性としては2つのルートが考えられる。一つには、肺胞へ感染したウイルスが組織破壊を起こした際に、血管バリアーが壊れウイルスが血中にもれてしまう可能性。もう一つの可能性として、増殖したH5N1が積極的に血液中に入っていく可能性が考えられる。これまでインフルエンザウイルスがウイルス血症を起こしたという報告(1-4)もあるが、逆には発症前には血液中にウイルスを検出できないとする報告(5-7)もある。季節性インフルエンザウイルスに関してはウイルス血症を起こす可能性は低く、万が一起こしたとしても極めて短い期間であろう。H5N1では16人中9人がウイルス血症を起こしたとう報告がある。
⑫	FDA (Nov 2009)	Guidance for Industry Recommendations for the Assessment of Blood Donor Suitability, Blood Product Safety, and Preservation of the Blood Supply in Response to Pandemic (H1N1) 2009 Virus DRAFT GUIDANCE	2009H1N1インフルエンザウイルスによるウイルス血症については、限られた情報しか得られていないが、米国その他の地域において、輸血により季節性インフルエンザに感染した事例は報告されておらず、同様に輸血により2009H1N1インフルエンザに感染した事例は報告されていない。現時点において、2009H1N1インフルエンザに感染した無症候状態の者の血液や血清から2009H1N1インフルエンザウイルスは分離されていないが、研究は継続中である。輸血による2009H1N1インフルエンザ感染の可能性は不明のままである。

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MEDICAL INTELLIGENCE

HUMAN INFLUENZA INFECTION WITH PROVED VIREMIA*

Report of a Case

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ALTHOUGH there is some indirect evidence in the medical literature that viremia may occur during human influenza infections[1,2] the isolation of this virus from a patient's blood has not, to the best of my knowledge, been reported. The present communication describes the isolation of influenza virus Group A, Type 2, from both the blood and throat secretions of a patient with clinical manifestations of influenza.

CASE REPORT

Three days before admission a 40-year-old physician noted the onset of severe headache and generalized malaise. He did not believe that he was febrile. This continued until the day before admission, when he noted shaking chills, and the temperature rose to 104°F. At that time he felt confused and somewhat restless. On the morning of admission to the Peter Bent Brigham Hospital he had several bouts of shaking chills followed by fever.

At 32 years of age an episode of fever accompanied by chest and arm pain had resulted in hospitalization and a diagnosis of idiopathic pericarditis. At the time of discharge from the hospital the electrocardiogram had returned to normal. No immunization against influenza had been taken at any time.

Physical examination revealed no abnormalities other than an enlarged thyroid gland. Throughout the hospital course the lungs were clear to percussion and auscultation, and the heart sounds were normal, without any murmur or rub.

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The white-cell count was 10,650, with 60 per cent neutrophils, 23 per cent band forms, 15 per cent lymphocytes, 1 per cent monocytes and 1 per cent basophils. The hematocrit was 51.5 per cent, and the corrected erythrocyte sedimentation rate 4 mm. per hour. Throat culture grew alpha-hemolytic streptococci and *Diplococcus pneumoniae*. Sputum culture revealed alpha-hemolytic streptococci, *D. pneumoniae*, *Staphylococcus aureus* and *Escherichia coli*. Febrile agglutination tests, including typhoid O and H, paratyphoid A and B, *Brucella abortus* and *Proteus X-19*, were negative.

The temperature on admission was 99.4°F., and on the following day the patient experienced shaking chills and the temperature rose to 103.6°F. On the 3d and 4th days the temperature did not rise over 100.2°F., but on the 5th day he again had a shaking chill accompanied by a rise in temperature to 102.8°F. From the 6th day on, he had only low-grade fever and noted a gradual diminution of the marked malaise. He became completely afebrile on the 9th hospital day. An electrocardiogram obtained on the 3d hospital day showed flattened T waves in Lead V_6. A chest film on the 4th day revealed that the heart was somewhat enlarged in transverse diameter as compared with films taken after the previous bout of pericarditis. Fluoroscopy on the 6th day showed enlargement of the left ventricle. Five days later an electrocardiogram revealed a normal T wave, and a chest film showed normal cardiac size and shape.

On the 2d hospital day specimens were obtained for attempts at viral isolation. These included clotted blood, a throat wash and a stool specimen that was made up as a 10 per cent suspension in tissue-culture medium.

Aliquots of each of these specimens (0.2 ml.) were inoculated into both primary grivet monkey-kidney and human-amnion cell cultures as well as into the amniotic sac of 7-day-old embryonated hens' eggs. Inoculated cultures and uninoculated controls were tested for hemadsorption 8 or 10 days after inoculation. The amniotic fluid of inoculated and control eggs was harvested on the 5th to the 7th day after inoculation and tested for hemagglutinating factors. Table 1 summarizes the results of the laboratory tests involved in the original isolation, passage and reisolation of the agent.

Hemagglutination-inhibition tests with guinea-pig red cells were used as a means of identifying the agent present in amniotic fluid that had been inoculated with passage material. Tests in accordance with the hemagglutination-inhibition technic recommended by the Committee on Standard Serological Procedures in Influenza Studies[3] revealed no inhibition with antibody to prototype influenza A (PR-8) and B (Arizona) strains. Antiserum to influenza Group A, Type 2 (Asian), inhibited hemagglutination by both the blood and the throat agent in dilutions up through 1:180. Subsequently, the hemagglutinating agent thus identified was reisolated in eggs from both blood and throat specimens (Table 1).

Using blood obtained on admission as an acute-phase and blood obtained 2 weeks after admission as a convalescent-phase specimen, an assay was done for hemagglutination-inhibition antibody. The blood agent, throat agent and a standard strain of influenza virus Group A, Type 2, were all used as antigens. The acute-phase blood in dilutions as low as 1:5 did not inhibit hemagglutination with any of the 3 agents, whereas the convalescent-phase serum inhibited hemagglutination by blood, throat and standard antigen against influenza virus Group A, Type 2, in dilutions through 1:80.

TABLE 1. *Summary of Attempts to Isolate Influenza Virus from Various Materials.*

MATERIAL	RESULTS OF 1ST ATTEMPT*						RESULTS OF 2D ATTEMPT				
	ORIGINAL INOCULATION			1ST PASSAGE			ORIGINAL INOCULATION		1ST PASSAGE		
	human amnion	*grivet monkey kidney*	*egg*†	*human amnion*	*grivet monkey kidney*	*egg*†	*grivet monkey kidney*	*egg*†	*grivet monkey kidney*	*egg*†	*identification*‡
Throat	–	–	+	–	+	+	–	+	+	+	Influenza virus Group A, Type 2
Blood	–	+	+	–	+	+	+	+	+	+	Influenza virus Group A, Type 2
Stool	–	–	–	–	–	–	–	–	–	–	
Controls	–	–	–	–	–	–	–	–	–	–	

*Virus recovered.
†7-day-old embryonated hens' eggs, inoculated intra-amniotically.
‡By hemagglutination-inhibition tests.

An attempt was made to determine the amount of virus present in both the blood and throat specimens. Serial half-log dilutions were made of both specimens and then inoculated into eggs. Only the undiluted specimens were positive.

DISCUSSION

Repeated isolation of influenza virus Group A, Type 2, from a specimen of this patient's blood gives clear evidence that on occasion viremia may occur in influenza caused by this agent. A report of the detection of influenza virus from the liver, spleen, kidney, heart and lymph nodes of patients who died during the outbreak of Asian influenza in 1957[1] strongly suggests that the virus might enter the circulation during the course of the disease. The report of isolation from human urine by another investigator[2] affords additional evidence that viremia may occur. Hamre, Appel and Loosli[4] have shown that viremia may be established in mice after intranasal inoculation of influenza virus Group A (PR-8). A low titer of virus was sporadically demonstrable in the blood only of mice that had a high viral concentration in their lungs. These investigators suggested that viremia in mice might arise as a result of a spillover from the pulmonary focus. If one accepts such a mechanism in human influenza infection, it seems logical to look for viremia at the peak of pulmonary infection rather than at an earlier stage.

Loosli and his co-workers[5] have shown that in mice given airborne influenza infection, both pneumonia and antibody to the agent develop. When mice are given influenza antibody intraperitoneally at the time of viral inoculation pneumonia but not active immunity develops. These results are interpreted by Hamre, Appel and Loosli[4] as indicating a need for generalized spread of virus to antibody-forming sites before active immunity can occur. If this assumption is correct and if it also applies to human influenza infections one can hypothesize that viremia of at least some degree occurs in all patients with influenza infection in whom antibody to the agent develops.

To my knowledge there have been no previous reports of the isolation of influenza virus from the blood of patients. Two papers[6,7] have noted unsuccessful attempts at such isolation. The possibility also exists that there are many unpublished accounts of other unsuccessful attempts at such isolation. One unpublished study by Gresser and Dull[8] includes 9 patients with the clinical signs and symptoms of influenza, with isolation of influenza virus from the throat washings of 7 and without isolation of the virus from any of the blood specimens when the washed leukocyte fractions were tested. It is difficult to account for the differences between the present case and the previous cases studied. As previously suggested,[7] the viremia in influenza may be quite transient, and by chance the present specimen was obtained at the proper time. Another unlikely possibility is that the present patient had some immunologic defect. However, both this patient and those studied by others[7] had no detectable hemagglutination-inhibiting antibody at the time blood was drawn for viral studies. Furthermore, medical history, antibody response to the agent isolated and serum electrophoretic pattern give no indication of any abnormalities of the present patient's immune mechanism. Minuse and his associates[7] suggest that nonspecific inhibitors in the patients' blood may have accounted for their failure to demonstrate influenza virus in blood specimens. The possible lack of such inhibitors was not investigated in the present patient.

SUMMARY

Influenza virus Group A, Type 2, was isolated and reisolated from both the throat washings and blood specimens of a forty-year-old physician hospitalized with shaking chills and fever. A significant rise in hemagglutination-inhibiting antibody was demonstrated both to the agent isolated from the patient and to the standard influenza antigen. Although the report of isolation of influenza virus at autopsy from many of the organs of influenza patients gives evidence of a viremia phase in human influenza, the present study is believed to be the first report of a direct isolation of influenza virus from a patient's blood.

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BRIEF RECORDING

Hemolytic Reaction after Novobiocin Therapy

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A SIX-year-old girl was seen at the United States Army Hospital, Fort Rucker, Alabama, with a chief complaint of mild jaundice and dark urine present for one day. The child had previously been seen by a civilian physician, five days before admission, because of sore throat and fever, with a maximum temperature of 105°F. At that time she had been thought to have pharyngitis, and novobiocin, 30 mg. per kilogram of body weight per day, was started by mouth. The fever subsided and the patient improved. However, on the day of admission she was noted to be mildly jaundiced and had been passing dark-brown urine.

Physical examination disclosed icteric sclerae and pale mucous membranes and conjunctivas. The throat was red, but no exudate was present. The remainder of the physical examination was negative.

The initial impression was that of hepatitis. A blood specimen revealed marked hemolysis on three different occasions, and a hemolytic reaction was suspected. The initial white-cell count was 4800, with a normal differential. The hemoglobin was 7.0 gm. per 100 ml., and the reticulocyte count 1.1 per cent. The blood urea nitrogen was 24.2 mg. per 100 ml. The remainder of the blood chemical findings, including the antistreptolysin-O titer, were within normal limits. A red-cell fragility test showed hemolysis at 0.50 per cent and ending at 0.00 per cent. A tourniquet test was negative. The platelet count was normal. Blood cultures were negative at ten days. During the first twenty-four hours in the hospital the hemoglobin dropped to 4 gm. per 100 ml. The urine was within normal limits except for a trace of bile and a positive test for hemoglobin. The direct and indirect Coombs tests were positive. The blood was Type O+, and two transfusions of this type of blood were given. Prednisone (Meticorten), 40 mg. per day, was started. After the two blood transfusions the hemoglobin rose to 10.2 gm. per 100 ml. The reticulocyte counts increased steadily from 1.1 per cent to a high of 10.2 per cent just before discharge on the fifteenth hospital day. The hemoglobin rose slowly from 10.2 gm. per 100 ml. after transfusions to a discharge level of 13 gm.

The urine cleared within two days after transfusion and institution of cortisone therapy, and the patient became essentially asymptomatic. She was discharged on the fifteenth hospital day with a final diagnosis of acquired hemolytic anemia.

It is possible that the hemolysis resulted from sepsis, but this is unlikely in view of the normal white-cell counts and the absence of fever during the hospital stay. This hemolytic reaction could also have been of the idiopathic variety, but novobiocin remains strongly suspected as the etiologic agent.

The patient has been seen on several follow-up visits, and the hemoglobin is holding steady at 13.5 gm. per 100 ml. The Coombs tests, direct and indirect, have returned to negative, and she is doing well.

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BY THE LONDON POST

Lord Nuffield — Pharmacy in Britain — Holiday Reading

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THE story of the life of Lord Nuffield, who died in August at the age of eighty-six, is like a fairy tale. Born in 1877, William Morris was educated in local schools until the age of sixteen, when, having shown some mechanical aptitude, he was sent to work in a bicycle shop in Oxford. Within a year he had borrowed £4, with which he opened a shop on his own account. He started by repairing bicycles, then he sold and raced them, and later he produced a model of his own. In his first six years of bicycle manufacturing he accumulated £2,000 of capital, and in another ten years, by the age of thirty-three, he had doubled that amount. By 1911 there were some 50,000 private motorists in Britain, and in the following year the Morris car appeared. During World War I the Morris works were turned over to war work, but at the end of hostilities motorcar production was started in earnest. In 1922 nearly 7000 cars were sold, and by 1925 the annual figure had risen to over 52,000.

His business success thus assured, he began to direct his attention to giving financial support to advance the study and practice of medicine, which, in fact, had secretly been his own first choice of career.

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BRITISH MEDICAL JOURNAL

Proved Viraemia in Asian Influenza (Hong Kong Variant) During Incubation Period

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Summary: During an outbreak of influenza specimens were obtained from 21 patients with influenza-like illnesses and from 29 healthy subjects in close contact with the patients. Throat washings from 12 of the patients were positive for influenza virus but virus was not detected from the blood specimens. One healthy contact became ill 12 hours after the specimens were obtained, and the virus was isolated from his blood and throat washings. The remaining contacts showed no clinical illness ; but the virus was isolated from the throat washings of four of them, with no viral isolation from the blood specimens.

Introduction

The occurrence of viraemia in influenza infection has been suspected after recovery of the virus from extrapulmonary tissues of man and animals (Hamre *et al.*, 1956 ; Kaji *et al.*, 1959 ; Oseasohn *et al.*, 1959). One of us (K. N.) reported isolation of Asian influenza virus from blood and throat-washing specimens of a physician suffering from an influenza-like illness (Naficy, 1963). Our further attempts to detect influenza virus from 18 proved cases of influenza were unsuccessful (K. Naficy, unpublished data). Stanley and Jackson (1966) showed that viraemia in influenza occurred in their human volunteers only during the first three days of the incubation period. We here report the successful isolation of Asian influenza virus, Hong Kong variant, from blood and throat-washing specimens of a patient who was in the incubation period, and an unsuccessful attempt to find viraemia in the same person and 21 other patients while demonstrating the clinical manifestations of influenza illness.

Materials and Methods

Subjects.—In mid-December 1968 we were informed of an outbreak of influenza illness among prisoners of the Tehran Ghasr Prison. The outbreak had apparently been present for a few weeks, during which period more than 200 prisoners had contracted the disease. Specimens were obtained from 21 patients in the first 24 hours of their illness, as well as from 29 healthy individuals who denied having had influenza-like symptoms in the two weeks prior to our visit. Both groups of prisoners gave informed consent to these procedures.

Specimens.—Throat washings and clotted and heparinized blood were obtained from all subjects. Sera from clotted blood were stored at −20° C. before use for serological tests ; throat washings and heparinized blood specimens were either inoculated within a few hours of collection or stored at −70° C. before inoculation. Second blood specimens were obtained three weeks later from only nine subjects.

Viral Isolations.—Each specimen was inoculated in a volume of 0·1 ml. into the amniotic sac of three 10-day-old embryonated hen's eggs, and incubated at 35°C. for 40 hours, then left at 4° C. overnight before harvesting the amniotic fluid. The fluid was tested for haemagglutinating activity ; if positive, passage was carried out allantoically, otherwise at least one blind passage was performed amniotically.

Haemagglutination and Haemagglutination Inhibition Tests.—These tests were carried out according to the standard technique modified for microtitration by four haemagglutination units of antigen and chick red cells.

Reference Influenza Virus, Hong Kong Variant.—Importation of Hong Kong variant influenza virus to Iran apparently occurred during the Eighth International Congresses on Tropical Medicine and Malaria, Tehran, 7–15 September 1968. During the congresses one-third of the participants contracted the disease, and several strains were isolated from them in our laboratories (Saenz *et al.*, 1969) ; these were confirmed by Dr. Pereira of the W.H.O. World Health Influenza Centre to be Hong Kong variant A2. One strain of these isolates—designated 30T—was used as a reference antigen.

Clinical Investigation.—Owing to the absence of any medical record in the Ghasr Prison, one of us (M. K.) made a daily visit to our subjects for six days and conducted clinical follow-ups.

Results

Clinical Manifestation.—Clinical manifestation of the disease consisted of fever, headaches, and generalized symptoms such as malaise, chills, anorexia, muscular pain, cough, sore throat, and chest pain in most of our patients, lasting from one to four days. No bacterial complication, encephalitis, or myocarditis was noted. All healthy subjects remained asymptomatic during the entire period of observation except one who developed fever and generalized symptoms 12 hours after the specimens were obtained.

Viral Isolation.—Twelve out of 21 throat-washing specimens obtained from the patients were positive for influenza virus either in the original inoculation or after the first passage. No virus was detected from the blood specimens of these patients in spite of two blind passages. Haemagglutination inhibition antibody determination in paired sera of seven patients revealed eightfold or greater rise both to the isolates and the reference antigen, except in one case. Table I summarizes these results in cases with positive viral isolations. No virus was isolated from the blood specimens of 28 healthy individuals who were in close contact with the patients and remained asymptomatic

TABLE I.—*Antibody Titres to Asian Influenza (Hong Kong variant) in 12 Patients with Positive Virus*

Patient's No.	Viral Isolation		Reference Antigen		Isolate	
	Throat	Blood	Acute	Convalescent	Acute	Convalescent
1	+	−	<1 : 8	1 : 64	<1 : 8	1 : 32
2	+	−	1 : 32	1 : 1,024	1 : 16	1 : 512
3	+	−	1 : 64	1 : 1,024	1 : 16	1 : 1,024
4	+	−	<1 : 8	1 : 128	<1 : 8	1 : 64
5	+	−	1 : 256	1 : 256	1 : 128	1 : 128
6	+	−	<1 : 8	1 : 512	<1 : 8	1 : 256
7	+	−	<1 : 8	1 : 1,024	<1 : 8	1 : 512
8	+	−	<1 : 8	N.T.	<1 : 8	N.T.
9	+	−	<1 : 8	N.T.	<1 : 8	N.T.
10	+	−	1 : 16	N.T.	<1 : 8	N.T.
11	+	−	1 : 256	N.T.	1 : 256	N.T.
12	+	−	N.T.	N.T.	N.T.	N.T.

N.T. = Not tested.

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during our six-day observation, but throat washings from four subjects were positive. One healthy subject developed clinical illness 12 hours after blood and throat-washing specimens were obtained. These were positive for influenza, and the blood isolates were sent to the World Influenza Centre, being confirmed by Dr. Pereira to be the Hong Kong variant. Reisolation of the virus from the original blood specimen was successful, but no virus was detected from the blood specimens obtained 12 and 24 hours after clinical manifestation. The paired sera of this case showed a 16-fold rise both to the blood isolate and to the reference antigen. Table II lists viral isolation and haemagglutination inhibition antibody of healthy contacts, with positive isolations.

TABLE II.—*Antibody Titres to Asian Influenza (Hong Kong variant) in 5 Healthy Contacts with Positive Virus*

Contact's No.	Viral Isolation		Reference Antigen		Isolate	
	Throat	Blood	Acute	Convalescent	Acute	Convalescent
1*	+	+	1 : 8	1 : 256	< 1 : 8	1 : 128
2	+	−	1 : 16	N.T.	< 1 : 8	N.T.
3	+	−	1 : 1,024	1 : 128	1 : 512	1 : 64
4	+	−	1 : 1,024	N.T.	1 : 512	N.T.
5	+	−	1 : 32	N.T.	1 : 16	N.T.

* Developed clinical symptoms of influenza 12 hours after obtaining specimen.

Discussion

Recovery of influenza virus from extrapulmonary tissue of man and animals was the first indication of the occurrence of viraemia during influenza infection (Hamre *et al.*, 1956 ; Kaji *et al.*, 1959 ; Oseasohn *et al.*, 1959).

Recovery of influenza virus from a patient's blood with clinical manifestations of the disease was the first report of proved viraemia in man (Naficy, 1963). Several other investigators, however, had failed to demonstrate viraemia during the clinical course of influenza infection (Kilbourne, 1959 ; Minuse *et al.*, 1962 ; K. Naficy, unpublished data). Stanley and Jackson (1966), using human volunteers, showed clearly that viraemia occurs during the incubation period and that the virus was not detected after the third day of infection. Our results demonstrate that in 12 out of 21 patients with clinical signs of influenza virus was isolated from the throat-washing specimens but none from their blood ; while in one patient—who proved to be in the incubation period at the time specimens were obtained—virus was obtained from both the blood and the throat washings. These results are in agreement with Stanley and Jackson's report and clearly explain accounts of unsuccessful attempts to demonstrate viraemia during the symptomatic phase of influenza infection.

The first successful report of the isolation of influenza virus from human blood, however, remains unexplained, since the isolation was made while the patient was symptomatic. Nevertheless, a review of the history of this patient showed that there had been two phases of clinical symptoms: (1) before admission and during the first two days of hospitalization, after which he became almost asymptomatic ; and (2) a second phase from the fifth day, when he again experienced fever and chills (Naficy, 1963). Thus it is conceivable that fever and chills on the fifth day of hospitalization marked the onset of his influenza, unrelated to his undetermined previous infection, and the specimens were obtained during the incubation period.

It should be noted that four healthy subjects from whom virus was isolated remained asymptomatic. Two of these had a high haemagglutination inhibition antibody titre (1 : 1,024) in their acute sera. Thus it seems that, in spite of high circulating antibody, local replication of the virus in the nasopharyngeal cavity takes place, and may play a part in spreading the infection.

We wish to thank Dr. Pereira of the W.H.O. World Influenza Centre for his help in confirming the Hong Kong variant of our isolates, and Dr. Jamshidy of the Ghasr Prison health centre for his co-operation.

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Medical Memoranda

Thrombotic Microangiopathy Associated with Squamous Carcinoma

British Medical Journal, 1969, **4**, 209–210

The association of malignant disease with thrombophlebitis migrans is well recognized, and in some instances the lesions of the veins may be the first indication of occult malignant disease (Sproul, 1938). Such patients may also have non-bacterial thrombotic endocarditis (MacDonald and Robbins, 1957). On the other hand, disseminated arteriolar and capillary lesions occur much less frequently and do not normally give rise to clinical manifestations (McKay and Wahle, 1955 ; Azzopardi, 1966). Recently we had the opportunity of studying a patient who presented with features of thrombotic thrombocytopenic purpura, and only at necropsy did it become clear that these were associated with widespread thrombosis of small vessels and recurrent carcinoma.

CASE REPORT

The patient was 56 years old when she was first seen in December 1963 complaining of rectal bleeding. This proved to be due to a rather poorly differentiated squamous carcinoma situated in the anal canal. Metastatic squamous carcinoma was also found in inguinal lymph nodes removed in a block dissection seven months later. After this, however, she remained well for nearly five years until bleeding occurred from the colostomy in November 1968. When admitted to hospital, after having symptoms for three days, she was severely anaemic and had a thrombocytopenia (Hb 3·7 g./100 ml., white cells 9,000/cu. mm., and platelets 65,000/cu. mm.). Blood transfusion brought some improvement in the haemoglobin level. Six days after admission, however, the platelet count was still only

Underlying diseases associated with pulmonary pseudallescheriasis include diabetes mellitus, leukemia, lymphoma, aplastic anemia, Cushing's disease, collagen-vascular diseases, and alveolar proteinosis. *P. boydii* may cause pulmonary infiltration (with or without cavitation) to occur and fungus balls to develop. However, to our knowledge, we report the first case of intrabronchial pseudallescheriasis. Moreover, we also report the first case of pseudallescheriasis in a healthy person who had no immunologic defects. Since *Pseudallescheria* species and *Aspergillus* species both produce septate hyphae and share some morphologic features, *Pseudallescheria* may be histologically misdiagnosed as *Aspergillus* in the absence of identification by culture [9, 10]. Although in our case the endobronchial biopsy findings were initially thought to be consistent with aspergillosis, the fungus was identified as *S. apiospermum* by culture.

Itraconazole therapy was administered after the fungus was identified since the MIC of this drug was lower than that of other drugs. However, the intrabronchial lesion persisted after 12 weeks of itraconazole therapy.

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Use of the Polymerase Chain Reaction for Demonstration of Influenza Virus Dissemination in Children

Most investigators believe that influenza virus does not usually induce viremia [1]. Although CNS, cardiac, and skeletal muscle complications have been described in relation to influenza, virus was successfully isolated from the blood and extrapulmonary organs in only a limited number of cases [1, 2]. We recently demonstrated with use of PCR that influenza A/PR/8 virus produces viremia in a mouse model during the acute phase of disease [3].

We searched for influenza virus in the blood and CSF of children with virologically confirmed influenza from 22 December 1994 to 26 March 1995 (table 1). Patients ranged in age from 6 months to 8 years; bronchiolitis was clinically diagnosed in four cases, bronchitis in five cases, and upper respiratory infection in six cases. No abnormal shadows were found in the lung fields on any of the children's chest roentgenograms. None of the children had a history of recurrent serious infectious diseases.

Serum hemagglutination inhibition titer of antibody to A/Kitakyushu/159/93 (H3N2) virus significantly increased (at least a fourfold increase from acute titer to convalescent titer) in 12 cases, it significantly increased to B/Mie/1/93 virus in five cases, and it significantly increased to both strains in two cases. Culture of throat swab specimens in MDCK cell suspension yielded H3N2 virus for 4 of 12 children. PCR and successive Southern hybridization were performed with primer sets for influenza A and B virus matrix gene as previously described [3, 4]. Influenza A and B viruses were detected by PCR in eight and two cases, respectively. However, blood fractions of virus could not be detected by PCR in any of the 14 cases (table 1).

Six children, including two epileptic patients with mental retardation, had convulsions during the course of our study. One child showed signs of somnolence. Because CNS infection was suspected in these cases, CSF was examined for a greater than normal number of cells and an increased protein concentration; however, pleocytosis was not detected, and the protein concentration was within normal limits. PCR was performed with these CSF samples, but they were negative for influenza A and B virus. Influenza virus was not isolated from blood samples or CSF.

This study has verified that viremia and transmission of the virus to the CNS cannot be easily detected among children infected with recent strains of influenza virus. We have previously shown that the PR8 strain of influenza A virus becomes viremic in immunocompetent mice [3]. Furthermore, we tentatively concluded that the virus enters the bloodstream through the infected alveolar septum. This hypothesis is supported by the finding that viremia does not occur when alveolitis is prevented by previous intraperitoneal administration of the antiserum to the virus. The fact that it was difficult to detect viremia among the children in our study might support this hypothesis since none of our patients had obvious pneumonia on the basis of chest roentgenogram findings.

In addition, we could not find any direct evidence that influenza virus invades the CNS of these infected children. Rantala et al. described the successful isolation of influenza B virus from the CSF of a child with febrile convulsions [2]. It might be possible that a certain strain of influenza virus induces systemic dissemina-

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Table 1. Use of PCR for detection of viremia in children with virologically confirmed influenza.

Age	Date of onset of influenza	Clinical sign; diagnosis	Temperature		Serum HAI antibody titer to influenza virus			Sample (d)*	Virus isolation	Results of PCR				
					Conv. Acute	Conv. Acute	Conv. Acute							
			°C	No. of days	H1N1	H3N2	B		Throat	Throat	PBMC	RBC	Plasma	CSF
6 mo	12/22/94	FC; bronchiolitis	40.0	6	<4 <4	<4 256	<4 <4	2	ND	ND	ND	ND	ND	–
8 y†	1/8/95	Convulsion; bronchitis	40.4	5	128 128	<4 1,024	32 32	0	–	–‡	–	–	–	ND
2 y	1/11/95	Bronchitis	40.2	7	<4 <4	<4 128	32 64	1	–	A§	–	–	–	ND
3 y	1/11/95	Bronchitis	41.5	5	<4 <4	<4 128	32 32	1	ND	ND	–	–	–	ND
3 y	1/12/95	FC; URI	39.5	7	<4 <4	<4 128	16 16	1	–	A§	–	–	–	–
2 y	1/12/95	Drowsiness; URI	39.8	5	8 8	<4 64	8 8	0	H3N2	A§	–	–	–	–
1 y, 5 mo	1/25/95	FC; URI	40.3	6	<4 <4	<4 128	<4 <4	0	H3N2	A§	–	–	–	–
9 mo	2/1/95	URI	40.1	11	<4 <4	<4 256	8 8	1	H3N2	A§	–	–	–	–
1 y	2/5/95	FC; bronchitis	39.0	3	<4 <4	<4 128	32 128	1	ND	ND	–	–	–	–
5 y†	2/5/95	Convulsion; bronchitis	39.2	5	<4 <4	<4 128	<4 128	1	–	A§	–	–	–	ND
1 y, 4 mo	2/9/95	Bronchiolitis	39.7	7	<4 <4	<4 128	<4 <4	5	H3N2	A§	–	–	–	ND
1 y, 4 mo	2/9/95	Bronchiolitis	38.5	8	<4 <4	<4 512	<4 <4	5	–	A§	–	–	–	ND
4 y	2/13/95	URI	40.0	5	<4 <4	256 256	16 2,048	4	–	B‖	–	–	–	ND
5 y	3/5/95	URI	40.0	2	<4 <4	256 256	32 128	1	–	B‖	–	–	–	ND
1 y, 7 mo	3/26/95	Bronchiolitis	39.6	7	<4 <4	<4 <4	<4 32	2	–	–	–	–	–	ND

NOTE. Conv = convalescent; FC = febrile convulsion; HAI = hemagglutination-inhibiting; ND = not done; PBMC = peripheral blood mononuclear cells; URI = upper respiratory infection.

* No. of days after the onset of illness.

† This patient had a history of intractable epilepsy and mental retardation.

‡ Negative for both influenza A and B viruses.

§ Positive for influenza A virus–specific sequences.

‖ Positive for influenza B virus–specific sequences.

tion if it is pneumotropic enough to cause pneumonia. Host factors should also be considered when investigating virus spread in immunocompromised individuals because one might expect them to have more serious illnesses [5].

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Detection of Influenza Virus RNA by Reverse Transcription-PCR and Proinflammatory Cytokines in Influenza-Virus-Associated Encephalopathy

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Eleven children with acute encephalopathy associated with an influenza virus infection were treated during the 1997–1998 influenza season. Reverse transcription-polymerase chain reaction (RT-PCR) assay was used to detect the viral genome in peripheral blood and cerebrospinal fluid (CSF) samples. The results were compared with those of control influenza patients without neurological complications. Viral RNA was detected only in the peripheral blood mononuclear cells of one patient with influenza-virus-associated encephalopathy (1 of 9; 11%) and in the CSF of another patient (1 of 11; 9%). RT-PCR was negative in the blood of all the controls, but the percentage of RT-PCR-positive samples in the two groups was not significantly different. Cytokines and soluble cytokine receptors in plasma and CSF were then quantified using an enzyme-linked immunosorbent assay. The CSF concentrations of soluble tumor necrosis factor receptor-1 were elevated in two patients and interleukin-6 (IL-6) was elevated in one patient with influenza-virus-associated encephalopathy. On the other hand, the plasma concentrations of IL-6 were elevated in four of nine patients. The number of encephalopathy patients who had elevated plasma concentrations of IL-6 100 pg/ml was significantly higher than that of controls ($P = .01$). In conclusion, the infrequent detection of the viral genome in the CSF and blood showed that direct invasion of the virus into the central nervous system was an uncommon event. Proinflammatory cytokines and soluble cytokine receptors may mediate the disease. The high plasma concentration of IL-6 could be an indicator of the progression to encephalopathy. ***J. Med. Virol. 58:420–425, 1999.***

KEY WORDS: influenza virus; encephalopathy; RT-PCR; interleukin 6

INTRODUCTION

Infection with influenza viruses can produce a spectrum of clinical responses ranging from a febrile upper respiratory illness to central nervous system (CNS) involvement with significant mortality. After the first human influenza virus was isolated in 1933, several examples of influenza-associated encephalopathy have been reported. Two specific types of acute encephalopathy are reported to accompany influenza infection: Reye syndrome and influenza-associated encephalopathy. Reye syndrome, which is a neurologic and metabolic disease with hepatic dysfunction and fatty accumulation in the viscera, often follows viral infections and the use of salicylate [Balistreri, 1996].

Influenza-associated encephalopathy, which occurs at the height of illness and may be fatal, has been described by many investigators [Dunbar et al., 1958; Flewett and Hoult, 1958; McConkey et al., 1958; Delorme and Middleton, 1979; Protheroe and Mellor, 1991; Murphy and Webster, 1996]. The cerebrospinal fluid (CSF) is usually normal, the brain shows severe congestion at autopsy, and histological changes are minimal [Murphy and Webster, 1996]. The pathogenesis of this CNS syndrome is, however, unclear. In regards to the viral pathogenesis, one explanation is that CNS complications may be caused by hematogenous transmission of the virus to the CNS, although the existence of viremia is disputed and isolation of the in-

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TABLE I. Clinical Features of Patients With Influenza-Virus-Associated Encephalopathy

Patient no.	Age (years)/ Sex	GCS	Convulsion	Cerebrospinal fluid		Serum			Mortality and morbidity
				Cell count (/μl)	Protein (mg/dl)	AST (IU/L)	ALT (IU/L)	NH_3 (μg/dl)	
1	2/F	11	Yes	2	24	92	24	18	Recovery
2	2/M	12	Yes	15	27	49	15	34	Recovery
3	2/M	13	Yes	0	13	26586	13879	74	Recovery
4	2/M	12	Yes	6	21	56	41	53	Recovery
5	3/M	3	Yes	0	57	18088	10472	50	Sequelae
6	5/M	3	Yes	6	20	1276	1667	37	Sequelae
7	6/F	3	Yes	NA	NA	32	14	NA	Recovery
8	6/F	11	No	0	10	39	17	NA	Sequelae
9	11/M	11	No	NA	NA	200	72	NA	Sequelae
10	11/M	11	Yes	3	23	35	13	21	Recovery
11	13/F	3	Yes	NA	NA	10510	3160	NA	Death

GCS, Glasgow Coma Scale; AST, aspartate aminotransferase; ALT, alanine aminotransferase; F, female; M, male; NA, not applicable.

fluenza virus from CSF is rare [Stanley and Jackson, 1969; Lehmann and Gust, 1971; Mori et al., 1997; Tsuruoka et al., 1997].

In the 1997–1998 flu season, 11 children with acute influenza-virus-associated encephalopathy were treated. Reverse transcription-polymerase chain reaction (RT-PCR) assay was used to detect the viral genome in peripheral blood and CSF samples. Several cytokines and soluble cytokine receptors were quantified in samples from encephalopathy patients. The presence of tumor necrosis factor-α (TNF-α), soluble tumor necrosis factor receptor 1 (sTNF-R1), interleukin-1β (IL-1β), and IL-6 in CSF samples is important for predicting the clinical outcome and diagnosing encephalitis/encephalopathy [Ichiyama et al., 1996a, 1998]. However, little is known about the levels of these cytokines in plasma and CSF from patients with influenza-virus-associated encephalopathy. Study of the dynamics of these cytokines may improve understanding of the mechanisms of influenza-virus-associated encephalopathy.

MATERIALS AND METHODS

Patients and Controls

Eleven consecutive patients, aged 2–13 years (7 boys, 4 girls; mean age: 5.7 years), who were diagnosed with influenza-virus-associated encephalopathy between January and February 1998, were investigated. The clinical data for these patients are summarized in Table I. The level of consciousness was assessed using the Glasgow Coma Scale [Teasdale and Jennett, 1974; Reilly et al., 1988]. Influenza-virus-associated encephalopathy was defined as follows: (1) The patient had a preceding upper respiratory tract infection and an altered level of consciousness that could not be explained by other identifiable causes. (2) Reye syndrome according to the case definition of the Center for Disease Control and Prevention (U.S.A.) [Center for Infectious Diseases, 1991] was excluded. (3) Influenza virus RNA was detected in throat swabs with the RT-PCR assay. The serum hemagglutinin inhibition titer of antibody to H3N2 virus increased significantly in all 9 patients in which it was measured, at least fourfold from acute to convalescent titers.

Twenty-nine control patients aged 1–15 years (13 boys, 16 girls; mean age: 3.8 years) with influenza virus infections without any neurological complications were also studied. In all the control patients, the diagnosis of an influenza virus infection was also confirmed by the detection of viral RNA in throat swabs.

Samples

Peripheral blood samples from the patients and controls were collected in standard blood tubes containing ethylenediamine tetraacetic acid (EDTA). Plasma, peripheral blood mononuclear cell (PBMC), and erythrocyte fractions were isolated from 1 ml of whole blood by Ficoll-Paque (Amersham Pharmacia, Uppsala, Sweden) density centrifugation at 400 × *g* for 30 min at room temperature. The PBMC and erythrocyte fractions were washed twice with phosphate-buffered saline (PBS), resuspended in 200 μl of PBS, and stored at −70°C until use. CSF was obtained from patients with influenza-virus-associated encephalopathy and stored at −70°C.

RT-Nested PCR

For PCR aimed at the NS gene, sense primer NS3 (GGTGATGCCCCATTCCTTGA; positions 108–127) and antisense primer NS4 (ATTTCGCCAACAATTGCTCC; positions 486–505) were used in the first round. Primers NS1 (GAGGCACTTAAAATGACCAT; positions 249–268) and NS2 (CTCTTCGGTGAAAGCCCTTAG; positions 465–485) were used in the nested PCR reaction. These oligonucleotides were designed from the highly conserved region of the influenza A/PR/8/34 NS gene sequence [Buonagurio et al., 1986].

RNA was extracted from each sample using a QIAamp viral RNA kit (QIAGEN, Hilden, Germany), using a silica-gel-based membrane that binds RNA. The RNA extracted from 200 μl of each sample was eluted in 50 μl RNase-free water. Ten microliters of this solution were used for cDNA synthesis immediately after denaturation for 2 min at 80°C. The reaction buffer (final concentrations, 10 mM Tris-HCl [pH 8.3], 50 mM KCl, 1.5 mM $MgCl_2$), NS3 sense primer (25 pmol), deoxynucleoside triphosphates (0.5 mM final

concentration), 200 U Moloney murine leukemia virus reverse transcriptase (Gibco-BRL, Rockville, MD), and dithiothreitol (50 mM final concentration) were added to a final volume of 20 μl. After incubation at 37°C for 60 min, 5 μl of this solution were added to 45 μl of PCR mixture containing NS3 and NS4 primers (25 pmol each), 1.5 U of Taq DNA polymerase (TaKaRa Taq; Takara Syuzou, Otsu, Japan), and the same reaction buffer as used in the RT reaction. Amplification was carried out in a TP-240 thermal cycler (Takara Syuzou). The PCR program consisted of a 1-min preincubation at 94°C followed by 30 cycles of 1 min at 94°C and 20 sec at 62°C. Nested PCR was performed after transferring 1 μl of the first-round PCR product into a new PCR reaction mixture containing the nested primers under the same conditions. The nested amplification product, which was expected to yield a 237 base-pair sequence, was analyzed by electrophoresis through 1.2% agarose in a Tris-acetate-EDTA gel stained with ethidium bromide. Because the sequences of the designed primers are highly conserved, both influenza A and influenza B viruses were detectable (data not shown).

Synthesis of Positive Control RNA

A first-round PCR fragment, consisting of nucleotides 108–505 of the NS gene, was cloned into the pGEM-T plasmid (Promega). RNA transcripts were synthesized from the purified recombinant plasmid with T7 RNA polymerase (the Riboprobe in vitro transcription system; Promega) and diluted serially in diethyl pyrocarbonate-treated water. Ten-fold dilutions were tested by RT-PCR, and the detection limit was established reproducibly.

Enzyme-Linked Immunosorbent Assay for Cytokines and Soluble Cytokine Receptors

The concentrations of TNF-α, sTNF-R1, IL-1β, and IL-6 were determined with commercial sandwich-type enzyme-linked immunosorbent assay (ELISA) kits (IL-1β kit, Genzyme, Cambridge, MA; TNF-α, sTNF-R1, and IL-6 kits, R&D Systems, Minneapolis, MN). These assays were carried out according to the supplier's instructions. Sample values were determined from a standard curve.

Statistical Analysis

Data were analyzed using Fisher's exact test. A level of $P < .05$ was considered significant.

RESULTS

Sensitivity of RT-PCR

To determine the sensitivity of our RT-PCR assay, dilutions of synthesized RNA transcripts of the NS gene were prepared (Materials and Methods) and used for the RT-PCR assay. A minimum of three copies per 50 μl PCR reaction mixture could be detected (Fig. 1).

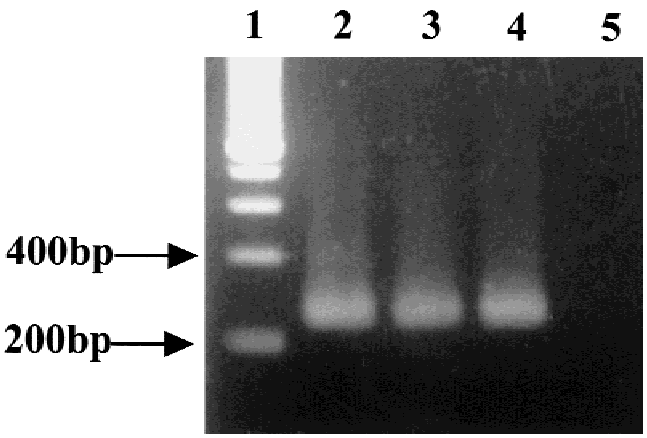

Fig. 1. Sensitivity of the reverse transcription-polymerase chain reaction (RT-PCR) in detecting influenza virus NS gene. **Lane 1**, 200 bp DNA marker ladder; lanes **2–4**, 3×10^2, 3×10^1, 3 copies of NS gene, respectively; **lane 5**, no template control.

Detection of Influenza Virus RNA

RT-PCR was carried out using blood samples (plasma, PBMC, erythrocytes) from the patients and controls, and CSF samples from the patients (Table II). Viral RNA was detected only in the PBMCs of one patient with influenza-virus-associated encephalopathy (1 [patient 9] of 9; 11%) and in the CSF of another patient (1 [patient 8] of 11; 9%). Viral RNA was not detected in plasma or erythrocytes from any of the patients. RT-PCR was also negative with all the blood samples from the controls. The percentages of RT-PCR positive blood samples in the two groups were not significantly different. The detection of viral RNA was not associated with any clinical features or the outcome, although the number of positive patients was small.

Concentrations of Cytokines and Soluble Cytokine Receptors

The levels of TNF-α, sTNF-R1, IL-1β, and IL-6 in the CSF of the patients with influenza-virus-associated encephalopathy are shown in Table III. The concentrations of TNF-α and IL-1β in the CSF were all below the detection limits. The CSF concentrations of sTNF-R1 and IL-6 were elevated in two and one patients, respectively, out of seven with influenza-virus-associated encephalopathy.

The levels of TNF-α, sTNF-R1, IL-1β, and IL-6 in the plasma of the patients with encephalopathy are shown in Table IV. The plasma TNF-α concentrations were all below the detection limits. In the nine patients with influenza-virus-associated encephalopathy, the plasma concentrations of sTNF-R1, IL-1β, and IL-6 (particularly IL-6 ≥ 100 pg/ml in three patients) were elevated in two, two, and four patients, respectively. The number of influenza-virus-associated encephalopathy patients who had elevated concentrations of IL-6 ≥ 100 pg/ml was significantly higher than that of the controls ($P = .01$) (Table V). There were no significant differences in the numbers of patients and controls with el-

TABLE II. Results of RT-PCR in Patients With Influenza-Virus-Associated Encephalopathy

Samples	Patients	Controls
Throat swab	9/9	29/29
Plasma	0/11	0/29
PBMC	1/9	0/29
Erythrocytes	0/9	0/29
CSF	1/11	ND

RT-PCR, reverse transcription-polymerase chain reaction; PBMC, peripheral blood mononuclear cells; CSF, cerebrospinal fluid; ND, not done.

TABLE III. Cerebrospinal Fluid Concentrations of TNF-α, sTNF-R1, IL-1β, and IL-6 in Patients With Influenza-Virus-Associated Encephalopathy

Patient no.	TNF-α (pg/ml)	sTNF-R1 (pg/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)
1	NA	NA	NA	NA
2	NA	NA	NA	NA
3	<15	1196	<4	324[b]
4	<15	2934	NA	<31.2
5	<15	1848	<4	<31.2
6	NA	NA	NA	NA
7	<15	555	<4	<31.2
8	<15	433	<4	<31.2
9	<15	553	<4	<31.2
10	<15	635	<4	<31.2
11	NA	NA	NA	NA
Normal range	<15	836 ± 402[a]	<4	<31.2

TNF-α, tumor necrosis factor-α; sTNF-R1, soluble tumor necrosis factor receptor 1; IL, interleukin; NA, not applicable.
[a]Mean ± SD.
[b]Underscores represent the level considered abnormal.

TABLE IV. Plasma Concentrations of TNF-α, sTNF-R1, IL-1β, and IL-6 in Patients With Influenza-Virus-Associated Encephalopathy

Patient no.	TNF-α (pg/ml)	sTNF-R1 (pg/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)
1	NA	NA	NA	NA
2	NA	NA	NA	NA
3	<31.2	2232	<8	860[b]
4	<31.2	810	30.2	18.2
5	<31.2	702	<8	<12.5
6	NA	426	<8	<12.5
7	<31.2	760	<8	<12.5
8	<31.2	869	<8	100
9	<31.2	>5000	<8	1295
10	<31.2	745	<8	<12.5
11	<31.2	270	21.1	<12.5
Normal range	<15.6	1020 ± 495[a]	<4	<12.5

TNF-α, tumor necrosis factor-α; sTNF-R1, soluble tumor necrosis factor receptor 1; IL, interleukin; NA, not applicable.
[a]Mean ± SD.
[b]Underscores represent the level considered abnormal.

TABLE V. Comparison of the Percentage of Patients Exhibiting Plasma Cytokines

Cytokines (pg/ml)	Patients (%) (n = 9)	Controls (%) (n = 29)	P
TNF-α	0 (0)	0 (0)	1.00
sTNF-R1	2 (22)	1 (3)	.13
IL-1β	2 (22)	2 (7)	.23
IL-6	4 (44)	12 (41)	.58
IL-6 (≥100)	3 (30)	0 (0)	.01

TNF-α, tumor necrosis factor-α; sTNF-R1, soluble tumor necrosis factor receptor 1; IL, interleukin.

evated concentrations of TNF-α, sTNF-R1, or IL-1β (Table V).

The concentrations of cytokines and soluble cytokine receptors in the CSF and plasma were not associated with any clinical features in the encephalopathy patients. In terms of mortality and morbidity, two patients who had cytokines in both CSF and plasma recovered without sequelae (patients 3 and 4).

DISCUSSION

Viremia is unusual in influenza virus infection [Murphy and Webster, 1996], although the virus is occasionally isolated from the blood [Stanley and Jackson, 1969; Lehmann and Gust, 1971]. Even when the RT-PCR assay is used, influenza RNA is detected only occasionally in blood samples from influenza patients [Mori et al., 1997; Tsuruoka et al., 1997]. In our study, viral RNA was detected infrequently in blood from patients with encephalopathy and never in blood from the controls. Viremia may be as rare in patients with influenza-virus-associated encephalopathy as it is in patients with influenza infection. Alternatively, the virus might be present in low titers in the blood.

Human influenza A viruses are reported to be neurovirulent in mouse models. Mice infected with influenza A viruses by intracerebral inoculation developed a meningoencephalitic condition [Nakajima and Sugiura, 1980; Sugiura and Ueda, 1980, Takahashi and Yamada, 1995]. Previously, PCR assay for detection of the herpes simplex virus genome in CSF was shown to be useful for virological assessment of patients with herpes simplex virus encephalitis [Kimura et al., 1991, 1992; Ando et al., 1993]. If influenza virus replicates in the brain tissue in a similar way to herpes simplex, then RT-PCR assay should also be a useful tool for analyzing influenza-associated-encephalopathy. A recent Japanese study detected viral RNA frequently in the CSF from patients with influenza-associated-encephalopathy [Fujimoto et al., 1998]. In that study, the RT-PCR assay of five of seven patients seen in the 1996–1997 influenza season was positive. RT-PCR was not undertaken on blood samples. In the present study, we established an RT-PCR assay to detect influenza virus RNA. Using this highly sensitive method, it was found that the RT-PCR assay was positive in only 1 of 11 CSF samples from patients with influenza-virus-associated encephalopathy. This result shows that although viral replication may occur in the CNS, it is an uncommon event.

It is not known why the frequency of detection of viral RNA differed in the two studies. One possibility is that the rate of CNS invasion differs according to the epidemic virus, although we have little information re-

garding to the respective capacity of 1996–1997 and 1997–1998 season viruses to induce encephalopathy.

Many cytokines and soluble cytokine receptors are considered important mediators of inflammatory responses, and their levels increase in CSF or plasma during infectious inflammatory disorders of the CNS, primarily meningitis [Mustafa et al., 1989; Chavanet et al., 1992; Glimåker et al., 1993; López-Cortés et al., 1993; Aurelius et al., 1994; Ichiyama et al., 1996a, 1996b, 1997, 1998]. We also reported previously that elevation of TNF-α, IL-1β, and IL-6 in the CSF indicates acute encephalitis/encephalopathy, rather than febrile convulsions mimicking acute encephalitis/encephalopathy [Ichiyama et al., 1998]. Previous studies showed that sTNF-R1 is the natural homeostatic regulator of the action of TNF-α, and that the level of sTNF-R1 is a better indication of the true biological activity of TNF-α than the level of TNF-α itself [Duncombe and Brenner, 1988; Englemann et al., 1990]. In the present study, the CSF concentrations of sTNF-R1 and IL-6 were elevated in two and one of seven patients, respectively, with influenza-virus-associated encephalopathy. It is not clear why sTNF-R1 and IL-6 were not always detected in the CSF. The inflammation of the CNS may be mild, so that inflammatory cytokines cannot be detected. Alternatively, influenza-virus-associated encephalopathy may have a different pathogenesis. In the influenza B virus mouse model of Reye syndrome, intravenous inoculation of the virus caused a nonpermissive viral infection of vascular endothelial cells of the brain and damage to the blood–brain barrier that resulted in acute encephalopathy without inflammation [Davis et al., 1990]. In an autopsy case of human herpesvirus 6 encephalopathy, human herpesvirus 6 viral antigens were detected only in the vascular endothelium of the brain and no inflammation was observed [Ueda et al., 1996]. These observations suggest that vascular endothelial infection is part of the pathogenesis of acute encephalopathy. Toxic factors and metabolic disorders, including hereditary enzymatic deficiency, are other possibilities.

The number of influenza-virus-associated encephalopathy patients who had elevated concentrations of IL-6 $\geq$ 100 pg/ml in plasma was significantly higher than that in the controls in our study. Monocytes and lymphocytes produce IL-6; however, it is particularly interesting that IL-6 is also produced by the vascular endothelium. IL-6 plays an important role in host responses to infection and induces hepatic protein synthesis, including C-reactive protein and fibrinogen, during the acute phase response [Heinrich et al., 1990]. Recently, it was reported that IL-6 affected the permeability of the blood–brain barrier in rats [Saija et al., 1995; Farkas et al., 1998]. In human neonates, IL-6 is thought to play a role in hypoxic-ischemic brain damage [Martín-Ancel et al., 1997]. It is possible that the systemic reaction to IL-6 contributes to the development of the influenza-virus-associated encephalopathy. Previous studies have described how IL-6 plasma concentrations are useful in the early diagnosis of neonatal infection [Messer et al., 1996; Panero et al., 1997]. Our results suggest that IL-6 plasma concentrations might also be useful in differentiating influenza-virus-associated encephalopathy.

In conclusion, the infrequent detection of the viral genome in CSF and blood indicates that direct invasion of the influenza virus into the CNS is an uncommon event, and suggests that systemic cytokines or vascular involvement may be indirectly responsible for the encephalopathy. A high plasma concentration of IL-6 may indicate progression to encephalopathy. However, the precise mechanism of the illness remains unknown. Further studies should explore the disease mechanism and the clinical applications of these observations.

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8

Maintaining a Safe and Adequate Blood Supply in the Event of Pandemic Influenza

Guidelines for National Blood Transfusion Services

19 May 2006

World Health Organization

1 Rationale

Current global concern that an occurrence of pandemic influenza may be imminent is based on recent experiences with avian influenza H5N1. However, a pandemic could also be caused by another influenza virus with the same pandemic potential.

Transfusion support is an essential component of clinical medicine, with transfusion being life-saving in many acute situations and many chronically ill individuals receiving regular transfusion therapy. It is therefore critical that national blood transfusion services (BTSs) recognize the potential impact of pandemic influenza on their blood supply systems and put contingency plans in place to ensure the maintenance of core services in the event of such a pandemic.

2 Recommendations

The Blood Transfusion Safety programme, Department of Essential Health Technologies, World Health Organization, proposes the following precautionary principles to national blood transfusion services to ensure the safety and adequacy of national blood supplies in the event of pandemic influenza.

1 Ensure the inclusion of the blood transfusion service in the national influenza contingency planning body.

2 Establish a mechanism for the blood transfusion service to receive regular, up-to-date epidemiological information on the spread of influenza in the country.

3 Develop a blood transfusion service contingency plan, which is reviewed constantly, regarding:
- Risk of transmission of influenza by blood transfusion
- Temporary loss of blood donors resulting in a reduced supply of donated units of blood
- Temporary loss of staff
- Changes in the clinical demand for blood and blood products.

4 Work with national health authorities, hospitals and other responsible bodies to determine expected blood usage during any pandemic and to plan blood collection activities accordingly.

5 Provide advice and guidance to all staff to minimize the risk of exposure, including the provision of prophylaxis, as appropriate and in accordance with any specific requirements in the national contingency plan.

3 General considerations

To ensure an effective and appropriate response to any pandemic, each blood transfusion service must ensure that it has a specific organizational contingency plan in place for this, and other major incidents. It should also ensure that it is actively involved in national contingency planning for pandemic influenza. While national planning often focuses on the early detection, verification and containment of infection, the implications for other aspects of health care of the rapid spread of an acute and severe infectious disease must be considered; blood transfusion support is such an example.

In the case of blood transfusion activities, contingency planning must include both planning for continuity in the supply of blood and blood products, and an awareness of probable changes in the demand for blood and blood products. In addition, in situations where containment measures are likely, or already in place, the blood transfusion service needs to ensure that blood collection activities do not compromise containment.

Central to planning is the inclusion of the blood transfusion service in the national contingency planning body. This ensures that the blood transfusion service is informed as early as possible about any possible emerging infections and the subsequent intended actions. This information can then be used as part of the service's own contingency planning to consider issues related to blood donation, possible cancellation of donation sessions in areas where cases have been identified and any potential changes in local or national requirements for blood and blood products. The blood transfusion service can plan effective responses only if this information is available to it as early as possible. In addition, any planned changes in the allocation or provision of health care nationally in response to any pandemic situation need to involve the blood transfusion service so that it can plan its activities to match planned national needs.

A major factor in the ability of a blood transfusion service to maintain an adequate safe blood supply is its overall structure and organization in terms of the number of its blood donation sites or sessions, the number of blood collection teams and the number of blood donors or potential donors within the catchment area of each site or session. In countries with more isolated population groups, for example, it is possible to focus collection activities in certain areas where any pandemic infection would take longer to infiltrate, increasing overall activity at these sites, within reason, to cover potential losses at sites closer to or in the middle of areas in which pandemic infection is more likely or already present.

Additional safety measures for the health and safety of staff will need to be introduced.

4 Risk of transmission through blood transfusion

The risk of the direct transmission of influenza via blood or blood products is extremely low. There are no published reports of the transmission of influenza viruses by blood transfusion in humans or in animal models**.** The transmission of a respiratory virus by transfusion is unlikely to result in an infection in the recipient except in the most extreme cases where the viral load is particularly high.

Importantly, a major assumption in all current international influenza pandemic contingency planning has been that infection with the emerging virus leads to moderate to severe respiratory illness. The incubation period for human influenza viruses in general is short: i.e. 2 to 3 days (range 1 to 7 days). However, with influenza A (H5N1), the median time between exposure and the onset of illness is 3 days (range 2 to 4 days). The early symptoms of influenza are very similar to those of most other respiratory viruses and, as part of the national blood donor selection guidelines, anyone who is symptomatic is not permitted to donate blood. It is not possible definitively to rule out any theoretical risk of the transmission of influenza through blood transfusion. In practice, however, the risk of this occurring is very small. Importantly, the risk of transfusion transmission is significantly less than that of contracting influenza from direct exposure through the airborne route of transmission. Further research is required to assess the level of viraemia in asymptomatic patients and the consequent risk of transfusion transmission.

5 Temporary loss of blood donors and the impact on the blood supply

As infection spreads through any population, the number of blood donors available at any one time decreases. This is due to infection in the donors themselves; infections in the families and contacts of donors; restrictions on movements, including blood collection activities in areas where outbreaks have been recorded; and the unwillingness of some individuals to donate due to a perceived risk of infection through being in close contact with others.

Infection in blood donors

Infection in the general population results in a decrease in the number of blood donors available. At any one time, up to 25% of donors could be lost due to infection. To mitigate this, the blood transfusion service should inform donors about the importance of maintaining an adequate national blood supply throughout any pandemic, but should also educate and inform donors about influenza, routes of transmission and signs and symptoms of infection. Specifically, donors should be informed of the importance of:

- Not donating blood if the donor is feeling unwell
- Reporting immediately to the blood transfusion service any illness within a specified time following donation
- Resuming blood donation on resolution of infection, after an appropriate time following complete resolution of symptoms.

It is critical that blood collection activities continue, but on a targeted basis, identifying and attracting low risk donors with the aim of maintaining blood collections at the required level.

Infection in families or contacts of blood donors

Donor numbers may decrease due to infections among donors' families and contacts rather than donors themselves, often because of time requirements in caring for infected individuals. In such a situation, donors would ineligible to donate as they would have been exposed to known infected individuals and might be at

an early stage of infection themselves. The losses of donors in this category may be higher than in those actually infected and cumulative losses of up to 50% may occur.

In addition, donors who have been vaccinated against influenza or have taken other prophylaxis may have to be deferred due to the prophylaxis administered. The duration of deferral should be determined within blood transfusion service contingency planning.

Restrictions in blood collection activities

As cases emerge, various strategies may be implemented in the attempt to prevent a pandemic ensuing. The current WHO Pandemic Influenza Draft Protocol for Rapid Response and Containment, updated draft 17 March 2006, outlines a containment strategy based on the rapid identification of potential or actual emerging disease and containment at source, using a number of approaches including vaccination, restriction of social interactions of infected individuals with non-infected individuals, and restrictions on movements into and out of areas where infections have been confirmed.

The containment strategy may impact directly on blood collection activities by limiting the ability of donors to attend donation sessions and, more importantly, by preventing mobile blood collection teams from visiting certain venues or areas. In addition, collection staff may either be exposed unnecessarily to infection or even contribute to the spread of infection. Thus, at certain times in certain areas, blood collection activities are likely to be limited significantly. Contingency planning for this is essential. Alternative strategies are needed to enable the rapid switching of collections from area to area, avoiding high risk areas and concentrating on educating and motivating donors and potential donors in low risk areas. Effective public awareness campaigns on the need for blood donation should run continuously throughout any pandemic.

Public and blood donor awareness

As with any major issue affecting the general population, ignorance or misinformation may deter individuals from donating blood through fear of exposure to an increased risk of infection. The blood transfusion service should address these issues by providing simple, clear information about the need for blood and the safety of the donation process. This information should be disseminated continuously throughout the pandemic, using all available media.

6 Loss of staff working in blood transfusion services

As infection spreads through any population, blood transfusion service staff will be at equal risk to the rest of the population of acquiring infection, in the absence of any specific preventive interventions. The loss of staff is highly likely to affect blood transfusion service activities such that, directly or indirectly, the blood and blood products available for release for clinical use will be limited. Depending on the organization and structure of the blood transfusion service, activities could be reorganized from site to site as the pandemic moves across the country and as staff become ill and then recover and return to work. The overall loss of staff at any one time is hard to predict but, in severe cases, staffing levels may fall by 50%, although a loss of around 30–35% is more likely.

7 Changes in the clinical demand for blood and blood products

A reduction in the clinical demand for blood and blood products during any pandemic phase may result from specific contingency planning involving the overall provision of health care. A reduced demand may also result from a reduction in healthcare provision due to a fall in staffing numbers resulting from influenza in healthcare professionals and should be anticipated. Planned or forecast changes in demand should be quantified and addressed in contingency plans, specifically to feed in to planned changes in collection activities.

National health authorities are responsible for contingency planning, in advance of any pandemic, for the reduced usage of blood and products resulting from planned reductions in healthcare activities. Planned reductions in blood usage by 20–50% can be achieved in situations where at least a certain amount of blood is used in routine, planned, but non-emergency situations which can be forecast with some degree of accuracy.

In situations where the blood supply is already limited and where most blood is used in acute/emergency situations (e.g. childbirth, severe infant anaemia due to malaria, trauma), planned reductions are not possible and no more than a 10% fall in demand can be anticipated. The demand may be reduced in situations where restrictions on the movement of individuals and social interaction are implemented, but this is likely to be minimal.

The demand for blood may decrease as infection spreads to healthcare staff. As they become ill and are unable to work, this will itself limit activities through the reduced ability of hospitals to function.

The guidelines in this document focus on the collection, processing and transfusion of blood and blood products. Nevertheless, the same basic principles can also be applied to the collection, processing and use of other banked products such as tissues and stem cells. However, the nature of a number of tissue products is such that the risk of transmission may be different from that of blood and blood products and specific individual risk assessments for the different tissue types and storage conditions must be undertaken.

These guidelines will be reviewed and updated as new information becomes available. They are compiled to provide a generic basis on which national heath authorities may wish to develop guidelines applicable to their own particular circumstances.

Planning for pandemic influenza: effect of a pandemic on the supply and demand for blood products in the United States

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BACKGROUND: Influenza causes episodic pandemics when viral antigens shift in ways that elude herd immunity. Avian influenza A H5N1, currently epizootic in bird populations in Asia and Europe, appears to have pandemic potential.

STUDY DESIGN AND METHODS: The virology of influenza, the history of the 1918 pandemic, and the structure of the health care and the blood transfusion systems are briefly reviewed. Morbidity and mortality experience from the 1918 pandemic are projected onto the current health care structure to predict points of failure that are likely in a modern pandemic.

RESULTS: Blood donor centers are likely to experience loss of donors, workers, and reliable transport of specimens to national testing laboratories and degradation of response times from national testing labs. Transfusion services are likely to experience critical losses of workers and of reagent red cells (RBCs) that will make their automated procedures unworkable. Loss of medical directors, supervisors, and lead technicians may make alternative procedures unworkable as well.

CONCLUSIONS: Lower blood collection capacity and transfusion service support capability will reduce the availability of RBCs and especially of platelets. Plans for rationing medical care need to take the vulnerability of the blood transfusion system into account.

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Influenza is a major cause of death. In typical years, it kills 30,000 to 50,000 people in the United States.[1] In the pandemic year 1957, the death toll was 70,000.[2] In the great pandemic of 1918, it was estimated that 675,000 died in the United States, 50 million worldwide.[3] The recent emergence of the H5N1 strain of avian influenza, "bird flu," raises concerns of a possible new pandemic.[4] Worldwide, 258 people have been infected, and 50 percent of these have died.[5] Projections based on the 1918 pandemic suggest that a new pandemic might infect and disable 30 percent of the US workforce at one time.[6] Direct effects on health care would include the inundation of hospitals with patients needing care, the loss of medical personnel and support staff to illness and absences necessitated by the illness of their loved ones, and the degradation of supporting economic and social infrastructure.

Blood transfusion is a critical part of modern health care, enabling the management of premature infants, congenital anomalies, trauma and burns, obstetric complications, and many complications of aging. Modern blood product provision and management is a highly developed, just-in-time logistic system, orchestrated at both the regional and the national level. It is susceptible to the loss of personnel and transportation at many points.

Effective planning for patients who may need blood during an influenza pandemic will require an understanding of how the blood supply system works and the likely ways that it will fail under the stress of pandemic disease with high rates of morbidity and mortality.[6] Dealing with the demand for blood will require clinical knowledge of the effectiveness of blood products and the way that pandemic disease will modify that effectiveness in individuals and cohorts. The appropriate use of a limited blood supply needs to be empowered. Such triage will raise ethical concerns and lead to confrontations with desperate individuals. If the care system is going to respond in a more than reactive way, health care providers must learn from the past and plan for the future.

BACKGROUND

Influenza

The genus *Orthomyxovirus* encompasses three species, influenza A, B, and C. Influenza A causes the majority of

clinically severe infections, usually manifesting as acute rhinotracheobronchitis with striking systemic symptoms, including high fevers and myalgias.[7] Secondary bacterial pneumonias are common. Facilitating the spread of influenza is the rapid growth of the viruses in the respiratory mucosa and the infectivity of viral droplets expelled by infected persons while coughing or sneezing, even before symptoms suggestive of influenza are present. Although people of all ages contract the infection, mortality is generally highest in patients at extremes of the age range, in infants or young children and in the elderly.

Influenza A strains are named based on the hemagglutinin and neuraminidase proteins on their surface: 16 described hemagglutinin and 9 described neuraminidase subtypes have been identified.[2] Viral replication is characterized by poor fidelity of RNA transcription, resulting in frequent mutations in the hemagglutinin and neuraminidase proteins present on the surface of the virus, thus allowing the virus to avoid recognition and elimination by the immune system. This process is known as "antigen drift." The influenza viral strains that have caused the majority of the yearly epidemics are the result of mutations in the H and N proteins, with three H and two N types predominating.

Viral strains with other H and N subtypes are found in animal populations, particularly in avian species, with occasional human infections reported in people who are in close contact with birds. In the absence of mutations that allow human-to-human spread of infection, these viruses will not cause epidemics within the human population. The frequent mutations that characterize the influenza replication process, however, and the possibility of coinfection of an animal or person with two strains, one human and one nonhuman, with consequent gene resorting, result in periodic exposure of humans to influenza viruses which have become capable of human-to-human transmission and to which they have little or no immunity. These larger changes in viral proteins are known as "antigen shift." The H5N1 subtype has been causing disease in various avian populations for the past 10 years, and an increasing number of humans have contracted it, almost exclusively those in close contact with fowl.[8] Several possible instances of human-to-human transmission have been reported within families, but there is no suggestion that the virus has mutated to a form that would allow epidemic transmission. The lethality of the H5N1 strain and the known genetic instability of influenza viruses, however, have raised concerns that another devastating pandemic could be on the horizon.

Blood-borne transmission of influenza has not been reported. Isolation of the influenza virus from the blood is unusual, but has been documented.[9,10] The H5N1 strain of influenza has been isolated from serum of an infected child in Vietnam[11] and plasma from an infected child in Thailand.[12] Although the short window period between exposure and symptoms and the historical rarity of documented viremia make influenza contamination of the blood supply likely to be at most an uncommon event, the recently reported cases of H5N1 isolated from blood specimens are concerning.

Treatment of influenza has been largely symptomatic, but there are both vaccines and drugs to limit infection and symptoms. Drugs such as amantadine and rimantadine, used in the past for prophylaxis, no longer appear to be effective, but the oral neuraminadase inhibitor oseltamivir can decrease severity and duration of symptoms if started within 48 hours.[13] There are two types of vaccines currently in use. The trivalent split vaccines are made from three strains of virus grown on the allantoic sac of embryonated hens' eggs, which are purified, concentrated, and then inactivated by the use of a detergent to disrupt or "split" the viral coat. A more recent development is the use of a cold-adapted live attenuated vaccine for intranasal administration. The vaccine synthetic process is slow, resource-intensive, and susceptible to contamination. Current research efforts involve other vaccine strategies, such as whole virus formalin-inactivated vaccines and monovalent split H5N1 vaccines with novel adjuvants that might prove more effective, but the production and distribution of vaccines in a timely fashion continue to present enormous logistical challenges. Therefore, despite our considerable knowledge of the virus and the pathophysiology of the infections it causes in humans and animals, there may be little actual protection available in the event of an acute outbreak.

The 1918 pandemic

The 1918 to 1919 pandemic peaked in the United States over 10 weeks in Fall 1918 when 5 to 40 percent of individuals in military bases and large cities were infected and 1 to 30 percent of these individuals died. The resulting deaths, estimated at 675,000, overwhelmed both the ability to provide care and to gather up and bury the dead.[14] Much of business and most of organized social life collapsed as functioning individuals struggled to respond to the many civic and personal crises. In places where the herd immunity was even lower, such as Western Samoa, infection was almost universal and mortality was 22 percent for the entire native population in a period of approximately 6 weeks.[15]

The blood supply system

Approximately 14.5 million units of red blood cells (RBCs) are collected in the United States every year, as well as approximately 1 million units of apheresis platelets (PLTs).[16] All of these products come from volunteer donors who must be recruited and offered comfortable and convenient opportunities to donate. It takes almost 1 hour to

donate, and the process is most efficient if it can be broken down into pieces in the context of large donor centers or their mobile blood drives. Large donor centers also take advantage of economies of scale by contracting the testing of collected blood to a handful of national testing laboratories. The blood supply system is reasonably efficient, providing RBCs for approximately $100 to $200 per unit, and has considerable surge capacity, as was demonstrated after September 11, 2001, but it is labor-intensive and absolutely dependent on the ability to assemble donors, maintain equipment, and move specimens and product.

The blood supply system is closely regulated by the US Food and Drug Administration. This oversight is designed to ensure that the production and shipping of blood components is conducted under current good manufacturing practices (cGMP), and compliance is enforced by the power of law. Blood collection services maintain staffs of quality control and compliance certification specialists who must know both blood banking and the intricacies of compliance, a process that consumes the time and efforts of many of the field's best workers.

Transfusion services

Collected blood is administered to approximately 2 million individual patients a year in the course of 3.8 million hospital visits and in outpatient infusion centers, dialysis centers, and home settings. The inventory management, final testing, and issuing of these blood products is the function of transfusion services. The transfusion service of the authors' hospital employs 40 technologists to provide round-the-clock coverage for a busy trauma center, a large transplant program, specialty surgery, a cancer center, and all of the other activities of a metropolitan university hospital.

The labor-intensive nature of transfusion services and the lack of pools of trained technicians are already problems for hospitals across the country. Highly skilled technicians are needed to cross-match blood, manage inventory, contact suppliers for products in demand, evaluate patients with antibodies, prepare further-modified products, and deal with the regulatory burden. National failure to educate an adequate number of technicians means the available pool is just adequate in the best of times. Automation of information and mechanical processes is slow, tied to evolving national standards, and poorly capitalized despite general recognition of system vulnerability.

The short shelf life of blood products means that inventory must be closely linked to immediate planned use. The size of local inventory is a compromise between ongoing day-to-day use and anticipated emergency need, with freshness sacrificed to build inventory and wastage the direct result of any lag in the demand for, or delivery of, blood products. In this sense, the management of liquid-stored cellular blood products is like that of fresh seafood or cut flowers.[17] Liquid storage systems can be made more effective, allowing somewhat longer storage and reducing losses, but the benefits are small.[18] Frozen storage is possible, but is expensive and labor-intensive and limited by process losses and low throughput.[19]

Transfusion medicine

In the United States, the use rate for RBCs is approximately 50 units per 1000 population.[20] Data concerning the recipients of the blood products are more difficult to obtain. A population-based study in Olmstead County covering the period from 1989 to 1992 found that 52 percent of RBC units were transfused into surgical patients.[21] More recent studies have suggested that medical patients are receiving a greater share of the transfused blood. Wells and coworkers[22] examined blood use in the north of England during two 14-day periods in 1999 and 2000 and found that 52 percent of RBCs were transfused for medical indications, with 41 percent for surgical patients and 6 percent for gynecologic or obstetric patients.[22] A study conducted of 175 randomly selected hospitals in France in 1997 revealed that 53 percent of patients receiving transfusions were on the medical wards including 30 percent on hematology wards.[23] Patients with neoplasms used 76 percent of PLT transfusions.

The academic specialty of transfusion medicine developed during and after World War II. As it has solved the technical problems of gathering and administering blood products, it has become more concerned with indications and safety. Recent attention has focused on the indications for RBCs, PLTs, and plasma and generally found that such products are overtransfused. Thus, Hebert and his colleagues[24] in the Transfusion Requirements in Critical Care (TRICC) study showed that the historic RBC transfusion trigger of 10 g of hemoglobin or 30 percent hematocrit (Hct) could be safely lowered in hemodynamically stable critical care patients with a 50 percent savings in blood product use and no worsening in mortality or morbidity. Studies have demonstrated a similar effect for PLT transfusion during leukemia induction therapy.[25,26] Dzik and Rao[27] have shown that plasma is administered frequently to prevent bleeding with invasive bedside procedures when the prothrombin and partial thromboplastin times are only mildly increased and that when plasma is administered, there is only rarely an improvement in the coagulation parameters.[28] In aggregate, these data suggest that many patients are exposed to blood products in situations in which they are unlikely to benefit. Enforcing blood use guidelines has worked best in conjunction with clear national standards, intensive education, and practice monitoring.

The coming pandemic: a projected scenario

The World Health Organization (WHO) has been following the H5N1 influenza activity in birds and people since the original outbreaks in Asia in the 1990s.[8] The disease has caused a high mortality in wild bird populations, has spread into domestic flocks, and has infected humans incidentally, in the context of close exposure to domestic poultry. In this form, it has infected 258 people over a decade and killed slightly more than half. There are no reports convincingly documenting human-to-human spread. The virulence of the virus might change if the virus evolves to allow efficient respiratory transmission in humans, but one could describe a likely clinical course based on past pandemics and the accumulated medical intelligence about the clinical course of the disease and the efficacy of drugs and vaccines.

The current planning model anticipates high infectivity, rapid worldwide spread in a matter of days to weeks, a broad range of severity of infection, rates of disability of 30 percent, and significant mortality. The basis for the model are described by the Interorganizational Task Force on Pandemic Influenza and the Blood Supply.[6]

Initial spread

The pandemic will probably start in Southeast Asia, where greater numbers of human beings live in close contact with domestic poultry than anywhere else on earth. There, national disease reporting systems may provide early warning and specimens for analysis by national and international reference labs. Attempts at quarantine may slow the spread of disease. Under the most optimistic of these scenarios, weeks might pass in a local epidemic phase, during which time knowledge of drug sensitivity might be established and some health care workers might be immunized with appropriate vaccines, with sufficient time to develop immunity. Under a less optimistic scenario, the virus might jump the species barrier and be transported in its human host on an airplane bound for the United States within a few days, causing a pandemic with very little warning.

Early response

With the recognition that an aggressive new influenza is threatening or active in the US, government, medical organizations, and the media will be deluged with questions and will be asked to provide information and guidance. Much of that guidance will be very general in nature, such as avoiding crowds, stocking up on canned food and water, and preparing for possible school closings and the reduced availability of goods and services. People who might have intended to donate blood will be presented with these other suggested activities and might be less willing to use potentially valuable gasoline to travel to a blood donation center or sit in a waiting room full of strangers.

Worsening crisis

As the number of individuals who are ill with influenza grows, two major effects will be seen. First will be an overwhelming utilization and depletion of hospital resources. Second will be the general social consequences of the accumulating loss of healthy workers.

Hospitals are the centerpieces of the national health care system, but a healthy population is only occasionally admitted to them and usually only for short stays. There were only 2.8 acute care beds per thousand members of the population in the United States in 2004.[29] More than 2000 hospitals were closed in the United States in the past two decades. By the time that one-half of 1 percent of the population needs medical care in an influenza pandemic, there will be two patients for every hospital bed in the country. Patients will be filling waiting rooms and lined up on gurneys in the emergency rooms, halls, and overflow areas of all public hospitals. There will not be enough staff to care for them, and medical and nursing students will be pressed into service to provide even minimal levels of care. Under these circumstances, it will not be possible to provide linens or remove waste. Caregivers will break down from exhaustion, frustration, and role confusion.

Hospital medical directors will have had to institute systems of triage. Care that can be deferred will have to be deferred. Care that is lifesaving and within the available resources will be undertaken. Care beyond the available resources should be deferred as well. Family members of patients can be expected to fight the decisions.

Blood donor centers will struggle to maintain donations of RBCs and PLTs. As increasing numbers of members of the population become ill or flee urban areas, regular donor roles will be decreased. Mobile blood drives to colleges will be lost as the education system closes, and blood drives to affinity groups such as churches will be limited as members' free time is filled caring directly for the sick. Mass media will still ask donors to come to blood centers, and moderate levels of donation will still go on. Getting blood tested for the usual infectious diseases will become more difficult as transportation and the productivity of regional reference laboratories degrades.

In the hospital transfusion services, maintaining staff for all work shifts will become increasingly difficult. Here, the loss of specific individuals, such as medical directors, supervisors, and lead technologists, will alter patterns of workflow that are written into policies and procedures and programmed into blood bank information systems. As remaining technologists are asked to assume responsibilities not usually their own, role confusion will occur. This will be especially evident in transfusion services

where a certain degree of obsessiveness is a basic job requirement, and the flexibility needed to deal with many kinds of stressful situations may be constitutionally lacking.

At some point early in the crisis, the FDA will issue guidance on how blood collection centers and transfusion centers may alter their function, because strict compliance with regulations and standards may no longer be possible. This was done at about midday on September 11, 2001, relaxing standards for training of individuals collecting blood, which allowed highly knowledgeable but not recently certified individuals to take part in blood collection.[30] The problem with such guidance is that it may not reflect the varied patterns of failure experienced by thousands of different transfusion services. It is difficult to imagine government guidance sufficiently broad to cover the situations of one hospital that cannot get a centrifuge repaired while another runs out of gloves.

At the height of the pandemic

By the time the pandemic reaches its peak, an estimated 30 percent of the population may be disabled by illness. Many more will be directly engaged in caring for ill family members. Emergency response personnel for other disasters (police, firefighters, electrical linemen, and communication workers) and regular workers involved in everyday commerce (production and transport of food and other basic supplies) will all be available in very reduced numbers. It will take longer to repair point failures in the electrical and telecommunications grids and water and sewerage systems. Secondary health crises will result. Even for healthy workers eager to do their duty, the increased demands of trying to find food and transport will degrade performance.

The blood system is very dependent on the rapid exchange of goods and services. Most notably, panels of reagent RBCs used for blood typing and antibody screening are collected by three companies in the United States and delivered under contract to thousands of hospitals every 3 weeks. It is likely that this part of the system will fail, that both blood collection centers and transfusion services would have to depend on forward-typing donor and patient cells with longer-lived monoclonal antibodies, and that the computer systems that manage labeling of blood products and routine blood typing will not work without this required data. The systems were often built without overrides for failures on such a basic level. The services will have to fall back on liquid cross-matching and paper records, more labor-intensive systems, precisely at a time when they are suffering from a severe labor shortage. PLTs are likely to be the first blood product where critical shortages are seen, but since PLT transfusions are given primarily to patients with hematologic malignancies, this will have its greatest impact on a subgroup of transfusion recipients.

Desperate situations frequently require desperate solutions, and it is often useful to know what other blood bankers have done in desperate situations in the past. In Sarajevo, during the siege in the early 1990s, artillery and sniper fire made the streets dangerous. Under these circumstances, citizens were instructed to send word to the blood center that they were willing to donate and teams of young people were trained to move quickly through the streets at night and collect individual blood units in basement shelters. Under such circumstances, the requirement for a predonation measure of the Hct was abandoned. The system worked well, providing the blood needs of citizens and the Bosnian defenders for 3 years (M. Haracic, NATO Blood Conference, 2000).

In Beruit, during the fighting in 1973 and again in 1982, the American University Hospital was isolated and cut off from supplies. Many workers could not get to the hospital because of the fighting, the bank was in danger of running out of blood bags, and gunmen were in the blood bank demanding rapid service for their comrades. Allam and his colleagues[31] describe the decision to collect only whole blood to reduce the use of bags and to issue group O un–cross-matched blood when workers were threatened.[31]

Finally, the US military has built up considerable recent experience with untested fresh whole blood in a variety of circumstances in Somalia, Bosnia, Kosovo, Iraq, and Afghanistan.[32,33] In Somalia, the force used its entire blood supply and needed more before resupply was possible. Blood was drawn from putative group O donors, type was confirmed by forward testing, and the blood given as fresh whole blood without further cross-matching. American soldiers are a relatively safe donor group in emergencies because they are routinely tested for human immunodeficiency virus and immunized for hepatitis B.

Recovery

After the peak of the pandemic, assuming there is only modest mortality, many of the absent workers will be returning to work. There will, however, be many competing demands for their efforts in restoring services and infrastructure. Some services will be relatively easy to restore, such as schools, where the buildings will be largely intact. Some teachers will need to be replaced and the social needs of grieving children will need to be addressed, but the mere act of opening the doors contributes in a major way to normality. Other parts of the social network will be harder to restore. Among the most difficult parts of a social system to rebuild will be those activities that are highly dependent on the specific knowledge of many individual people.[34]

The donor side of the blood system will recover relatively quickly. Its historic problem has been attracting donors, and after the pandemic, many survivors will be highly motivated to give. The return of manufacturing and transport will bring donor centers most of the supplies they need and, at a national level, temporary alternatives will be approved for the items that are not immediately available.

The transfusion service side of the blood system will be harder to rebuild. The loss of critical individuals and key bits of equipment will be random and each of the thousands of transfusion services will have unique problems. The chronic shortage of trained workers will only become worse. The national collapse in the supply of immunohematologic reagent RBCs will take time to replace, making more and novel work for limited staff. Many hospital blood bank computer systems, programmed by consultants before the disaster, will not have the flexibility to respond to the changing circumstances.

The need for well-trained and broadly experienced transfusion medicine specialists and blood bank technologists will be larger than the supply during and after the pandemic. This will create opportunities to expand training.

WHAT CAN BE DONE TO MINIMIZE THE DAMAGE AND MAXIMIZE OUR RESOURCES?

Planning for maintaining a blood system during a major influenza pandemic needs to address protecting personnel, recruiting donors, assuring access to supplies, preserving the function of equipment and facilities, and keeping a functioning management system.[35] Protecting personnel is best accomplished by vaccination and working for the designation of blood system workers as critical medical personnel who will be immunized or given oseltamivir chemoprophylaxis and access to rationed gasoline. Recruiting donors will require a clear public message and convenient and safe opportunities to donate. Access to consumable supplies, both perishable and durable, will require rethinking the limits of just-in-time logistics. High-volume supplies, such as test tubes and gloves, and short-lived supplies, specifically immunohematologic testing cells, will run out if a national disaster continues for many weeks. Deferred maintenance will degrade blood bank equipment and facilities. Modifying blood bank computer systems to allow workers to bypass steps involving temporarily nonexistent reagents will reduce role confusion and allow technologists to use the residual systems to manage inventory even as processes change. Ensuring the continuity of management will require both a plan and the ability to respond to an evolving situation.

Protecting all blood-system personnel, donors, workers, and managers is a major goal, and masks, social distancing, vaccines, and chemoprophylactics are the available tools. The utility of masks is unknown, and social distancing will be hard to achieve in many crowded facilities.[36] H5N1 research vaccines are available in very limited quantities, and stockpiles of oseltamivir are sufficient for only 1 percent of the US population.[2] Therefore, planning must start with the assumption of the temporary loss of 40 percent of all classes of personnel.

Loss of donors can partially be addressed with limited overcollecting in the early stages of a pandemic while there are still relatively full staffs at donor centers. This would allow limited stockpiling of RBCs and plasma and the provision of PLTs to continue while the pandemic takes shape. Because liquid RBCs are licensed for 5 to 6 weeks of storage, which is more than half of the 6- to 8-week length of the height of the 1918 pandemic in any given community, the potential of early excess collections to ease later shortages should not be lost.[2]

There is information supporting the use of RBCs beyond their current outdate. AS-1 RBCs were originally licensed for 7-week storage and worked as well at the end of that time as currently licensed 5-week CPDA-1 RBCs.[37] AS-3 RBCs have been tested for 8-week storage and could potentially be used for extended times as well.[38] PLTs are good in conventional storage for at least 7 days and were at one time licensed for that period. In emergencies, extending storage should be accompanied by careful inspection of bags for hemolysis of RBCs and loss of "swirling" in PLTs. Emergency rules for not discarding RBC units that have been out of the refrigerator for a little longer than 30 minutes should also be considered.[39]

Shortages of blood products will occur. Individual clinical services with the aide of blood bank medical directors will have to design triage schemes. Thus, a trauma service director might decide to set limits on which patients should be resuscitated given the restricted resources. In the United States, 10 to 15 percent of RBCs transfused are used in the setting of acute trauma.[20] A closer look at the recipients of these transfusions at one trauma center revealed that the majority of this blood was transfused into a very small fraction of the most critically injured patients: 3 percent of trauma patients received more than 70 percent of the RBCs transfused. Despite all efforts, the mortality for these massively transfused was 39 percent, half of this occurring in the first 24 hours.[40] In reduced circumstances of blood or nursing availability, mortality would be higher, care for such patients might be identified as futile, and resources could be directed elsewhere.

Chronic transfusion programs for genetic disease offer another example of a possible pattern for triage.

Individuals with thalassemia major do not make RBCs and need regular transfusions to survive. Efforts to transfuse them regularly need to continue through the crisis. On the other hand, individuals with sickle cell anemia on regular exchange transfusion programs to prevent stroke are exchanged monthly to reduce middle cerebral artery narrowing and increased flow velocity, which increased slowly when exchange was stopped.[41] More limited transfusion, rather than full exchange, would allow these individuals to get through a several-month-long critical period and free up blood and nurses for other efforts.

A significant fraction of blood products are given to patients with malignancies. The hematologic malignancies account for the majority of these products, in particular the PLTs. Slichter[26] has shown how the combination of lower PLT transfusion triggers and reduced PLT dosage can reduce PLT use by two-thirds. A UK study revealed that 33 percent of patients with solid tumors received at least one transfusion during the course of their therapy.[42,43] In the event of an influenza pandemic, careful consideration will need to be given to the risk:benefit ratio of cytotoxic chemotherapy with its attendant immunosuppressive effects. For instance, the result of adjuvant chemotherapy for Stage II breast cancer is a 9 percent net reduction in mortality in 5 years.[44] The acute mortality to be associated with being immunocompromised during an influenza pandemic is probably higher than that.

Transplant recipients, both solid organ and hematopoietic stem cell, are particularly susceptible to complications from influenza, with a relatively high rate of progression to viral pneumonia reported in some but not all series.[45,46] Because transplant patients are required to be in close contact with the health care system, more opportunities for exposure to the virus might exist. Infected transplant patients have been shown in some cases to shed viruses for extended periods. These safety concerns, both for the transplant patients and for the general population, might suggest to transplant program directors that a temporary suspension of transplant procedures would be in their patients' best interest. Given the likely shortage of hospital beds, health care workers, and ventilators in the event of a pandemic, this suspension would likely be encouraged by those in the health care system attempting to triage scarce resources. A temporary closure of the transplant program in Toronto was effected during the SARS epidemic, because of similar concerns.[47]

Transfusion service medical directors should make hospital medical directors and service chiefs aware of the limits of the blood supply. They should also reemphasize conservative blood use with its potential to prevent more than half of all transfusions. There are specific bits of knowledge that need to be reemphasized, such as the lack of benefit for RBC transfusion in helping patients get off of respirators and the evidence from a large consecutive series suggesting that lumbar punctures can safely be performed in children with low PLT counts.[24,48] Strict adherence to these guidelines will prevent the wasteful use of scarce resources, but there has been no effective educational intervention identified that will alter physicians' transfusion practices.[49] Empowering transfusion medicine experts to enforce these guidelines might be necessary so that blood products can be directed to those who need them the most.

In summary, influenza causes periodic pandemics. The H5N1 bird flu strain appears to be a potential agent for such a catastrophic event. Protection of the blood supply during a pandemic will require plans to protect donor center and transfusion service personnel, assure access to supplies and reagents, maintain equipment and facilities, and assure the continuity of management. Plans to manage blood use also need to be considered. Special attention needs to be given to the critical role of electronic information systems. The above insights on the nature of an influenza pandemic and on blood collection and use are offered as a contribution to the societal efforts that we collectively should be making to minimize the effect of a pandemic on public health and national welfare.

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Influenza viremia and the potential for blood-borne transmission

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Influenza is a major cause of morbidity and mortality in the United States and worldwide. The threat of pandemic influenza recently has gained prominent attention because of widespread infection of poultry with highly pathogenic avian influenza A (H5N1) and the potential for the virus to mutate into one capable of efficient human-to-human transmission. As of March 8, 2007, 277 human cases of H5N1 infection had been reported to WHO from Asia,[1,2] Eastern Europe,[3,4] and Africa,[3] mostly as a result of close contact between humans and infected birds, although rare, unsustained human-to-human transmission has been documented. If a change in viral characteristics were to allow efficient human-to-human transmission, rapid spread and a worldwide pandemic could result. The global spread of H5N1, continuing outbreaks in birds, and sporadic infections in humans have increased concern that a pandemic virus may emerge and cause an influenza pandemic. The possibility of an influenza pandemic has focused attention on the epidemiology and pathophysiology of influenza, including its potential for transmission through the blood supply.

An infectious agent which has a blood-borne phase and can be clinically asymptomatic has the potential to be transmitted by blood transfusion. Planning to ensure an adequate and safe national blood supply during a pandemic prompted consideration of the potential for transfusion-transmitted influenza. Notably, no cases of transfusion transmission of influenza have been documented to date. The risk of transfusion transmission has been assumed to be negligible based on the premise that viremia rarely occurs and does not occur without symptoms, allowing for deferral of potentially infectious blood donors. If these assumptions are incorrect, however, and influenza infection commonly involves a viremic phase, especially before the onset of symptoms, and if influenza-infected blood resulted in clinical illness, it would have implications for blood safety. To further examine this possibility, we surveyed the literature underlying these assumptions and propose methods of redressing gaps in our knowledge.

If influenza were transmissible by transfusion, blood product recipients, who include a high proportion of immunocompromised patients, might suffer increased morbidity and mortality. In bone marrow transplant populations influenza infection appears to be associated with approximately 25 percent mortality.[5,6] If influenza were deemed to represent a transfusion transmission risk, possible screening methods would likely rely on epidemiologic query or laboratory screening. With any screening there is a risk of impacting supply. Testing of the blood supply would also become particularly important if a pandemic were to be caused by a virus similar to the highly pathogenic influenza A (H5N1). Infection in humans has thus far resulted in approximately 60 percent case fatality ratio where the virus has been isolated outside of the respiratory tract, a factor not typically seen with seasonal influenza infections. Detection of influenza in the blood of H5N1 infected humans and in animal studies has occurred predominantly during symptomatic periods and at high viral titers. A positive test might result in discarding

ABBREVIATION: CSF = cerebrospinal fluid.

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the donated unit of blood, further endangering blood product availability at a time when donors may be scarce. Such additional sources of stress on the system would need to be taken into consideration when modeling the effect of a potential pandemic.

BACKGROUND

Influenza epidemiology

Seasonal influenza represents a significant contributor to overall mortality in the United States, particularly among those 65 years and older. The rates of illness and severe complications from seasonal influenza can vary substantially from year to year. In years of high influenza activity, weekly mortality incidence due to pneumonia and influenza can peak at more than 10 percent of all cause mortality in the United States (Fig. 1).[7] During the 1990s, a mean of 36,000 influenza-related deaths occurred per year, with more than 90 percent of deaths occurring in the elderly.[8] Estimates of annual influenza illness can range from 5 to 20 percent[9] with the highest incidence generally occurring in children.[10-14] For example, during a 1976 outbreak of A/Victoria in the Houston area, an 18 percent attack rate was documented, with rates in preschool children estimated to be more than 30 percent. Influenza attack rates can be much higher in populations with close contact, with documented attack rates of 42 percent for the crew of a US Navy ship.[15] In contrast to seasonal influenza where higher illness rates occur in children compared to adults, illness rates are generally high across all age groups in a pandemic. While mean illness rates are 6 to 7 percent in healthy adults in seasonal influenza, during a pandemic illness rates are estimated to be approximately 30 percent.[16-18]

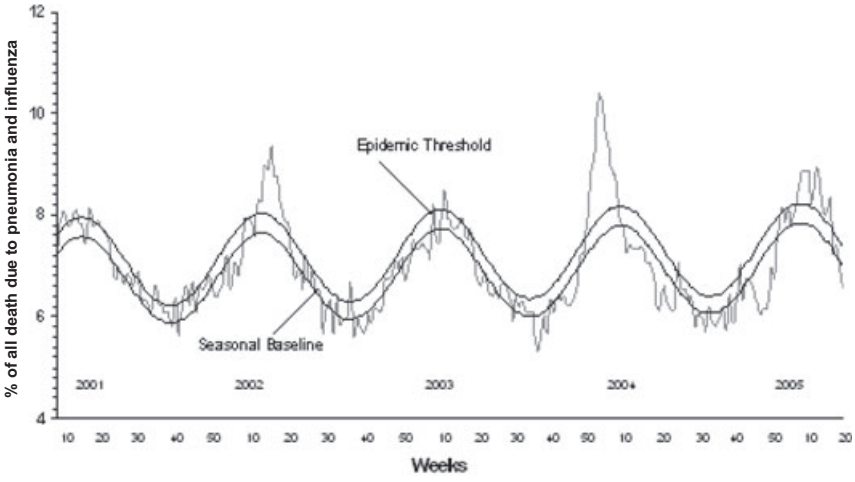

Fig. 1. Seasonal variation in influenza and pneumonia mortality for 122 cities, week ending May 21, 2005. The excess mortality seen most markedly in the winter of 2003 to 2004 is primarily attributable to influenza and secondary pneumonia associated disease.

Antigenic drift and shift and influenza reservoirs

Influenza A is a single-stranded, negative-sense RNA virus, with eight genome segments, each complexed to a nucleoprotein molecule in the mature virion. Antigenically important regions of the virus include the hemagglutinin, responsible for virus binding to sialic acid on host cells, and the neuraminidase, needed to detach newly replicated virus from host cells.[19] Antibody responses directed against the hemagglutinin protein can protect from influenza infection (reviewed by Potter and Oxford[20]), and antibodies against neuraminidase lessen disease severity.[21] There are two main types of influenza viruses, Types A and B. Type B viruses are found predominantly in humans. Type A viruses can infect multiple species including humans, birds, horses, pigs, and dogs. The primary reservoir for Type A viruses, however, is birds. Influenza A viruses are subtyped based on their hemagglutinin and neuraminidase. Both Type A and Type B viruses are included in the annual vaccine and can cause influenza epidemics.

During influenza virus replication, errors can occur and lead to genetic variation. Changes made to antigenic regions such as the hemagglutinin molecule are termed antigenic drift.[22] Antigenic drift allows individuals to have multiple infections with influenza viruses over their lifetime because antibody made against one influenza strain may not protect against distantly related drifted strains. Antigenic drift is an ongoing process and necessitates nearly yearly updates of the influenza virus strains included in influenza vaccines.

A more extreme version of antigenic variation in influenza viruses is antigenic shift, whereby genomic segments from different influenza virus strains reassort to create new combinations of gene segments.[23] The new virus can acquire a novel hemagglutinin or neuraminidase gene from the pool of 16 hemagglutinin and 9 neuraminidase genes.[24] Antigenic shift resulting in a strain capable of causing a human pandemic is a rare event. However, the two most recent influenza pandemic viruses arose through reassortment of human influenza A with avian influenza strains. Recent evidence suggests, however, that the 1918 influenza pandemic may have resulted from an avian influenza virus strain that acquired the capacity to infect humans by direct mutation rather than reassortment (Table 1).[25] A number of avian influenza strains have caused isolated infections in humans, ranging from conjunctivitis to influenza like illness and even death. Examples of contemporary strains that have caused more

TABLE 1. 20th century influenza A pandemics

Year	Name	Strain	Estimated mortality
1918	Spanish	H1N1	>20 million
1957	Asian	H2N2	70,000
1968	Hong Kong	H3N2	34,000

than one human case of infection in recent years include H5N1, first reported in 1997,[26] H9N2 reported in 1999,[27] H7N7 reported in 2003,[28] and H7N3 reported in 2004.[29] Of these, H5N1 represents the greatest current pandemic threat and will be discussed further below.

EVIDENCE FOR INFLUENZA VIREMIA IN HUMAN INFECTION

Naturally occurring human cases of influenza viremia

Relatively few studies have addressed the question of viremia caused by influenza infection, and most of these date back to the 1960s and 1970s. The first report of influenza viremia was made in 1963 by Naficy.[30] Influenza virus, Group A, Type 2 (Asian) was isolated from blood drawn on Hospital Day 2 (5 days after onset of symptoms) from a 40-year-old physician ill with fever, headache, and malaise. This report followed previous suggestions of viremia based on isolation of influenza virus from postmortem tissue samples taken from patients who had died of Asian influenza infection.[31] Subsequent to Naficy's publication, a single report in the literature describes influenza viremia in a naturally infected, asymptomatic patient.[32] Samples were prospectively obtained from 29 clinically well close contacts of 21 ill patients in a Tehran prison. Of these 21 ill subjects, 12 had influenza virus isolated from their throat washes either in the original inoculation or after the first passage in 10-day-old embryonated chicken eggs. No virus was detected in the blood specimens of these patients after two blind passages. The 29 healthy close contacts were clinically observed for 6 days and evaluated by both throat washes and blood specimens. Of 5 subjects who developed positive throat swabs, 1 had virus isolated from both blood and throat specimens collected while asymptomatic. This individual developed clinical illness 12 hours after these specimens were collected. Subsequent specimens collected at 12 and 24 hours after onset of symptoms were negative. The number of subjects studied was small, and laboratory contamination cannot be definitively ruled out, but 1 of 5 subjects observed from the time of influenza exposure who later developed documented natural influenza infection exhibited transient viremia. Seventeen symptomatic subjects with throat wash specimens position for influenza had negative blood cultures for influenza.

Before the report by Naficy in 1963, Minuse and colleagues[33] examined 7 college students hospitalized for Asian pandemic influenza in 1957. In spite of isolating virus from throat washes of 6 of these students and antibody evidence of infection in all 7, none of the blood specimens yielded positive results, although pooling of serially collected specimens may have diluted out a single positive specimen beyond the infectious dose for the chick embryo. Naficy, Minuse and Khakpour all used clotted blood specimens. In contrast, Poliakova and coworkers[34] isolated influenza virus from hemolyzed blood of 11 of 63 patients, all of whom were described as having moderately severe illness.[32] Ages of these 11 patients ranged from 9 months to 67 years, and virus was isolated most frequently on Day 3 after onset of symptoms (2 on Day 2, 5 on Day 3, 2 on Day 4, 1 on Day 5, 1 on Day 6, 1 on Day 7, and 1 on Day 13; 2 patients had virus isolated on 2 different days). Isolated case reports have demonstrated viremia in hospitalized patients either pre- or postmortem.[35,36] More recently, Tsuruoka and associates[37] used reverse transcription–polymerase chain reaction (RT-PCR) to identify genomic influenza RNA in peripheral blood mononuclear cells (PBMNCs) taken from 18 children aged 1 to 14 years with positive throat swabs for influenza during the 1992 to 1993 season in Japan. Three of the 18 samples were RT-PCR positive with NP and/or HA primers (subtype H3, the contemporary circulating subtype).[38] The NP gene sequence observed in 1 patient's PBMNCs was identical to that obtained from his throat swab, although the HA sequence of the other 2 isolates differed from the amplified throat nucleic acid by 3 to 9 nucleotides. This large number of nucleotide changes could signal either selection of a quasi-species of influenza virus that was not detected by culture or may represent contamination of samples. In addition, viral culture was not attempted in this study and thus did not assess presence of viable virus. During an influenza B outbreak in the 1994 to 1995 season, 5 of 17 red blood cell (RBC) and white blood cell (WBC) samples from 4 of 11 ill children yielded influenza B when cocultured with Madin–Darby canine kidney cells.[39] In this study as well, the nucleotide and amino acid sequences varied substantially between viruses isolated from throat and blood specimens. A number of studies searching for influenza viremia after the onset of illness have failed to detect virus, supporting the notion that influenza viremia is at most a rare event in the postsymptomatic period and if it exists is not generally sustained for long periods.[40-42]

Indirect evidence of influenza viremia has been suggested by reports of viral detection (isolation or PCR) from extrapulmonary sites including autopsy specimens of heart, kidney, brain, spinal cord, spleen, and liver of a pregnant 19-year-old woman who died as a result of A2/HongKong/8/68 infection.[43] Fetal heart tissue and amniotic fluid were also positive, as was the amniotic fluid of a 24-year-old woman who recovered from influenza A/Bangkok (H3N2) infection.[44] A series of autopsies

performed during the 1957 Asian pandemic also yielded positive results from spleen, lymph node, liver, kidney, heart, and tonsil.[31,45] Systemic dissemination of influenza virus is suggested in influenza-associated encephalopathy, although virus isolation from either blood or cerebrospinal fluid (CSF) from patients with encephalopathy and influenza is rare.[41,42,46,47] This may indicate that the presence of virus in the CSF is not required for the development of neurologic symptoms. Alternatively, viremia and possible infection of the CSF may precede the onset of encephalopathy. Influenza viremia has also been detected in a case of virus-associated myocarditis.[48]

Viremia after experimental human influenza infection

Experimental infection has resulted in isolation of virus from the blood of individuals infected by nasal inoculation.[40] With a cell line coculture system, virus was detected in nasal secretions of 1 of 15 subjects and was not detected in any blood samples. Samples were available for retesting from 4 subjects with existing antibodies against Asian influenza, with each of the subjects showing a fourfold or greater increase in titer following challenge. With a more sensitive egg inoculation culture system viremia was seen in all four subjects tested. Viremia preceded nasopharyngeal shedding of virus by approximately 1 day. The authors reported that symptoms began on the second or third day after challenge, which was 2 days after the initial viremia and 1 day after demonstration of virus in the nasopharynx. One of these 4 patients remained asymptomatic throughout the observation period (22 days) and was viremic through Day 3. These results imply that viremia can precede nasopharyngeal shedding of virus and can occur in asymptomatic individuals. Caution in interpreting these data is urged, however, because others have noted that a much larger dose of influenza virus is required to effect infection when intranasal inoculation is used compared to an aerosol route, and other investigators using a similar virus strain and methods were not able to detect viremia in 27 experimentally infected patients.[40,49]

Potential interaction of influenza with blood elements

Influenza typically does not cause major hematologic abnormalities aside from occasional lymphopenia, usually in the setting of a normal WBC count.[50-52] Thrombocytopenia in the setting of influenza infection has been noted in a number of case reports, often in the setting of concomitant antibiotic therapy, which can also affect the platelet (PLT) count.[50,52,53] Although influenza does not appear to cause major hematologic perturbations in patients, it has been shown to interact with blood elements.[54] Influenza has been shown to adsorb to PLTs and RBC with equal kinetics and to elute from PLTs more slowly and less completely than from RBCs.[55] Binding of live or dead influenza virus to PLTs in vitro caused morphologic signs of damage to the PLTs including swelling, ballooning, and fragmentation, with a pronounced decrease in PLT counts.[56] Intravenous infusion of influenza virus in rabbits caused transient thrombocytopenia to levels 50 percent of normal for 1 to 2 days' duration. In vitro experiments have shown that anti-hemagglutinin antibody causes lysis of influenza-treated PLTs via the classical complement pathway in the presence of autologous human serum.[57] Although hematologic abnormalities aside from lymphopenia are not typically observed during the course of influenza infection, the available data suggest that influenza can interact with WBCs, RBCs and PLTs. If viremia occurred primarily in presymptomatic or asymptomatic infection, transient disturbances of hematocrit or PLT counts would likely escape detection. Binding to cellular components within the blood in theory could facilitate influenza viremia, although evidence for the phenomenon is lacking in humans.

ANIMAL MODELS OF INFLUENZA INFECTION AND VIREMIA

Animal models may provide insight into the frequency of viremia after influenza infection, and the murine model of influenza infection has been used as a prototypic model of an antiviral immune response, defining which subsets of immune cells are important in protection from lethal lytic virus infection. Although the model has been widely used for study of the immune response to viruses[58] relatively few studies measure influenza viremia as a study outcome. In mice infected with influenza intravenously, viremia persists for approximately 1 week in immunocompetent mice and shows rising titers at 1 week in gamma-irradiated mice.[59] In mice intranasally infected with influenza, virus could be detected in RBC fractions on Days 1 through 5 but not in PBMNCs or plasma aliquots.[60] Virus disseminated widely to a number of organs, and viremia was blocked by treatment of mice with hyperimmune serum before infection. Local lung damage may play a role in the development of viremia. After intranasal influenza infection, no mice with histologically normal lungs developed detectable viremia, whereas 9 of 20 mice with lung congestion had influenza RNA detected in blood by RT-PCR.[61] Although the murine model has been widely used to study influenza infection, pathogenicity of influenza isolates in mice and men does not always show a correlation.[62] Simian models of influenza infection generally reflect lower pathogenicity than in humans,[63,64] and cynomolgus macaques show less systemic dissemination of highly pathogenic avian influenza virus than human hosts.[65] A model animal that may more closely parallel human influenza infection is the ferret. Human influenza

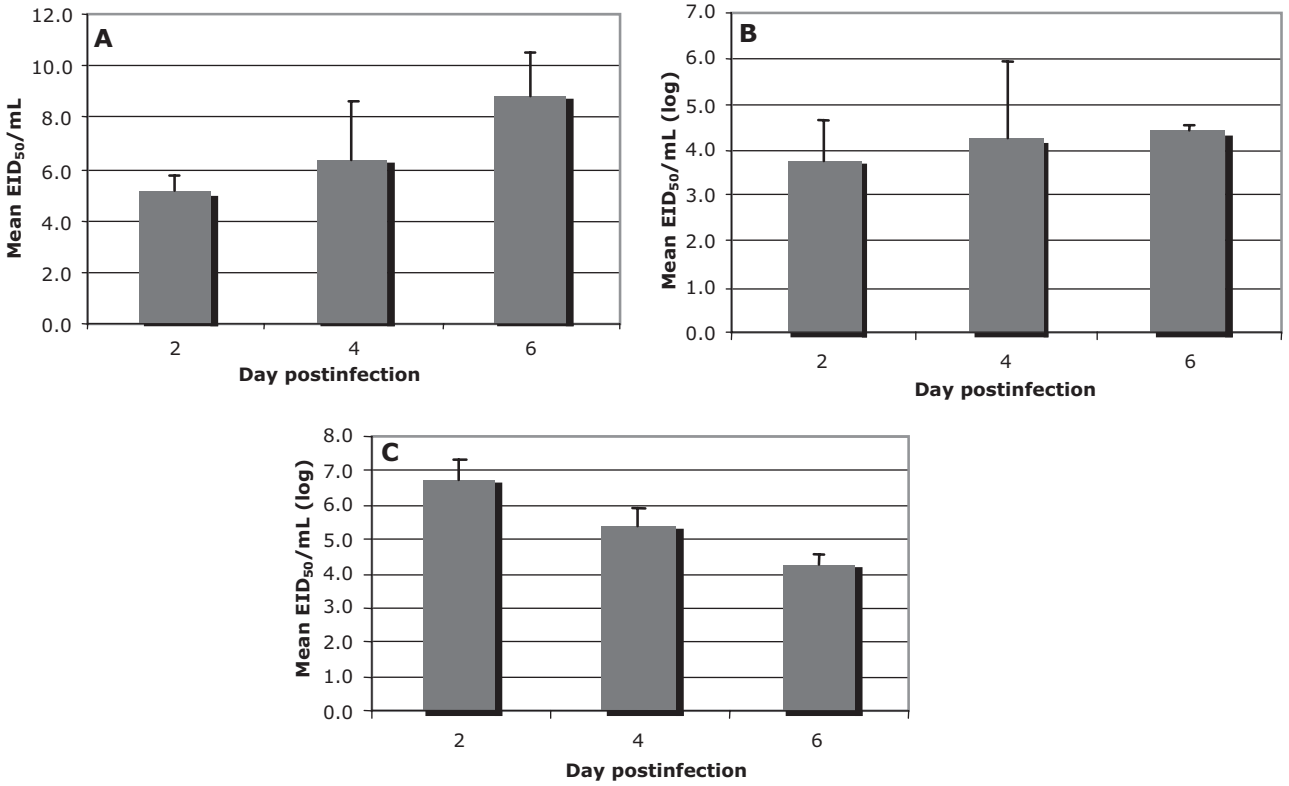

Fig. 2. Dissemination of H5N1 influenza A in ferrets. Viral loads in nasal turbinate (A), lung (B), and spleen (C) tissue from nine intranasally H5N1 (A/Vietnam/1203/2004) influenza A-infected ferrets. Ferrets were euthanized on Days 2, 4, and 6 postinfection, and tissues were immediately snap-frozen, homogenized, and inoculated onto embryonated hen's eggs for 50 percent egg infectious dose (EID_{50}) determination to assess viral load in the specified tissues.[83] Data shown represent the mean viral load in tissues from three ferrets per time point.

A strains with differential pathogenicity showed similar differential pathogenicity in ferrets, yet caused nonpathogenic infection in mice,[66] although ferrets may be more susceptible to influenza than humans.[67] Ferrets appear to be susceptible to highly pathogenic avian influenza H5N1 virus, developing severe symptoms with isolates from both fatal and mild human cases.[68] Multiple organ involvement is also observed in the ferret model of highly pathogenic avian influenza H5N1 infection. Representative original data from ferret challenge experiments with H5N1 influenza virus are included, revealing widespread productive viral replication in the brain, spleen, and upper and lower respiratory tract (Fig. 2). Ferret experiments were performed at the Southern Research Institute (Birmingham, AL) and were supported by the Canadian Institutes of Health Research.

HIGHLY PATHOGENIC AVIAN INFLUENZA A (H5N1)

Characteristics of transmission

Highly pathogenic avian influenza A (H5N1) has infected bird flocks in an almost worldwide distribution, yet transmission to humans remains rare, and human-to-human transmission has only occurred in isolated cases.[69,70] Tropism of the hemagglutinin molecule for its sialic acid receptor may influence the ease of infection and subsequent transmission of this avian influenza virus in humans. Avian influenza hemagglutinin binds to sialic acid linked to galactose by an α2,3 linkage (SAα2,3Gal), whereas human influenza hemagglutinin favors a sialic acid-galactose α2,6 linkage (SAα2,6Gal).[71,72] Upper airway epithelial cells in humans contain predominantly SAα2,6Gal, whereas SAα2,3Gal can be found in bronchiolar and alveolar cells.[73] Although clinical infection of humans with H5N1 virus rarely occurs, up to 10 percent of poultry workers in Hong Kong developed antibody responses to H5, implying that subclinical or abortive infection might occur in highly exposed subjects.[74] Exposure to live poultry was the only significant risk factor for transmission of H5N1 disease recognized in the 1997 Hong Kong H5N1 outbreak.[75] Mutations allowing use of the SAα2,6Gal receptor would likely be needed, in addition to other genetic changes, to transform the currently circulating avian H5N1 influenza virus into a human-tropic strain.

Disease manifestations in humans

Avian influenza H5N1 infection in humans causes increased morbidity and mortality compared to seasonal influenza infections in reported cases. H5N1 influenza infection in humans manifests as lower respiratory tract disease, consistent with the expression pattern of receptors for the virus.[2,70,76,77] Initial symptoms are similar to typical influenzalike illness, with fever (usually >38°C), cough, and dyspnea common.[2,77] Gastrointestinal symptoms also appear prominent in many case series, with one documented subject presenting with diarrhea but no initial respiratory symptoms.[78] An outbreak of H7N7 avian influenza was notable for conjunctivitis as a presenting symptom[28] although this has not been noted in H5N1 influenza illness. Although initial symptoms predominantly involve the respiratory system, multiple extrapulmonary tissues are affected by H5N1 avian influenza, including the gastrointestinal tract and prominent involvement of the liver and kidneys.[2,76,77] Encephalitis with virus isolated from the CSF has been reported in a child with no early pulmonary involvement.[78] Hematologic abnormalities are also prominent, with lymphopenia and thrombocytopenia frequently reported[2,76] and pancytopenia noted in one case series.[77] Marrow studies reveal hypoplastic marrow with a reactive hemophagocytic syndrome.[76] The involvement of multiple organ systems in H5N1 highly pathogenic avian influenza infection in humans suggests that subjects might show evidence of viremia. In fact, in two published studies measuring virus in the plasma or serum, virus was isolated, in the first case on Day 10 of illness (viral load not reported), and in the second a viral load of 85,000 RNA copies per mL was detected.[78,79] More recently, de Jong and coworkers[80] reported finding H5N1 viral RNA in the blood of 9 of 16 Vietnamese patients infected with avian influenza. These results suggest that viremia can occur at reasonably high levels and for prolonged periods in people with symptomatic H5N1 influenza infection. Based on limited data, it appears that symptomatic H5N1 influenza infection and infection with other new pandemic strains in humans may be more likely than currently circulating influenza A strains to result in viremia.[78,80] The risk of viremia during the incubation period of H5N1 infection or asymptomatic infection with other influenza A viruses novel to humans is unknown.

SIGNIFICANCE AND SUMMARY

With the advent of routine testing for multiple pathogens, the US blood supply has become increasingly safe. Many adverse events associated with transfusion are not related to transfusion-transmitted diseases, but rather immunologic effects (transfusion-related acute lung injury, ABO mismatch) or hemodynamic effects (transfusion-associated circulatory overload). Furthermore, the blood banks and test manufacturers have demonstrated that they can rapidly respond to interdict newly introduced infectious agents, as was seen in the response to West Nile virus introduction to the US.[81] Not all pathogens that infect blood donors, however, are successfully screened, with unknown impact on recipients. One potentially unrecognized pathogen in the blood supply is influenza virus. The incidence of viremia in blood donors is thought to be low, particularly for seasonal influenza, but has not been adequately studied. Older studies of influenza A experimental infection and one report of a naturally infected person suggest that viremia can occur before symptom onset. Even if influenza viremia occurs, the incidence is likely low during seasonal influenza outbreaks and would not pose a large risk to the safety of the blood supply.

Research projects are ongoing to address whether virus can be detected in blood collected during periods of transmission within the community. As part of the Retrovirus Epidemiology Donor Study II (REDS II), we are examining the incidence of influenza viremia in a retrospective cohort of blood donors, the REDS Allogeneic Donor and Recipient (RADAR) repository. The RADAR repository contains more than 120,000 whole-blood and plasma specimens that are linked to donor zip code and date of blood donation, but delinked from personal donor identifiers. Access to the repository will allow identification of samples collected during periods of widespread influenza activity in the community. Detection of influenza in the blood presents a challenge due to the lower viral loads found in blood compared to nasopharyngeal secretions. RBC or PLT fractions may contain more influenza than plasma or WBC fractions due to interaction with the hemagglutinin moiety of influenza.[57] We will first determine the blood fraction that contains the highest level of virus. Once the appropriate blood fractions are identified, samples from RADAR repository obtained during periods of known epidemic influenza A outbreak, and samples obtained during periods of very low influenza activity will be tested for the presence of influenza A RNA. This ongoing project will quantify the risk of occult influenza viremia in blood donors during periods of high-level transmission of influenza in the community. If viremia is detected then the issue of iatrogenic transmission of influenza by transfusion will need to be reassessed. In addition, further studies of people ill with seasonal and avian H5N1 virus infection are needed to evaluate the presence of viremia both by nucleic acid amplification and viral culture in comparison to viral isolates obtained from the respiratory tract to understand possible strain-specific and genetic features of viruses isolated from both sites.

Because pandemic influenza might be more likely to cause viremia and have higher pathogenicity than seasonal influenza, the risk of viremia during circulation of a new pandemic strain of influenza is more worrisome than

for possible transfusion-associated infection with currently circulating influenza, particularly given high rates in the population of antibody to current strains. More data is clearly needed to understand strain-specific characteristics that may predispose to viremia in infected persons. Based on limited available data, it appears that H5N1 virus infection and infection with newly circulating pandemic viruses in humans may be more likely than circulating seasonal human influenza viruses to cause a viremic phase. It is unclear how often asymptomatic infection occurs in avian influenza, although this may also be strain-specific as studies of human infection with earlier H5N1 strains found more mild or asymptomatic infections than studies of more recent H5N1 strains.[74,82] If and when a new pandemic appears, and the causative strain becomes evident, assuring the safety of the blood supply through study of donor viremia would be an important step. Widespread illness associated with an influenza pandemic among both donors and blood bank staff would stretch the ability of blood banks to provide a safe and adequate blood supply, and broad and indiscriminate screening based on exposure would only worsen potential product shortages. As part of pandemic planning, the resources to rapidly evaluate and implement screening measures for influenza viremia should be developed, allowing a rapid response to this potential threat to blood safety and availability.

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PATHOLOGY OF HUMAN INFLUENZA REVISITED

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Summary

The pathology of human influenza has been studied most intensively during the three pandemics of the last century, the last of which occurred in 1968. It is important to revisit this subject because of the recent emergence of avian H5N1 influenza in humans as well as the threat of a new pandemic. Uncomplicated human influenza virus infection causes transient tracheo-bronchitis, corresponding with predominant virus attachment to tracheal and bronchial epithelial cells. The main complication is extension of viral infection to the alveoli, often with secondary bacterial infection, resulting in severe pneumonia. Complications in extra-respiratory tissues such as encephalopathy, myocarditis, and myopathy occur occasionally. Sensitive molecular and immunological techniques allow us to investigate whether these complications are a direct result of virus infection or an indirect result of severe pneumonia. Human disease from avian influenza virus infections is most severe for subtype H5N1, but also has been reported for H7 and H9 subtypes. In contrast to human influenza viruses, avian H5N1 virus attaches predominantly to alveolar and bronchiolar epithelium, corresponding with diffuse alveolar damage as the primary lesion. Viremia and extra-respiratory complications appear to be more common for infections with avian H5N1 virus than with human influenza viruses. Further understanding and comparison of the pathology of human and avian influenza virus infections only can be achieved by directed and careful pathological analysis of additional influenza cases.

Keywords

influenza; human; pathology; pathogenesis

Introduction

An understanding of the pathology of influenza A virus infections in humans is important to improve diagnosis and to understand how these viruses cause disease. This knowledge also is important to evaluate animal models that adequately represent the disease in humans, and so to further unravel the pathogenesis and to test potential antiviral drugs and vaccines. We here review the pathology of human influenza A virus infections, both pandemic and seasonal, as well as that caused by infections with avian influenza A viruses such as H5N1 virus.

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Uncomplicated influenza

Human influenza A virus infections for which the pathology is described include H1N1, H2N2, and H3N2, which caused pandemics in 1918, 1957, and 1968, respectively [1]. Each time that a new subtype enters the human population it replaces the previously circulating subtype. The exception is the reintroduction in 1977 of H1N1, which has continued to co-circulate with H3N2.

Transmission of human influenza virus occurs by inhalation of infectious droplets or airborne droplet nuclei and, perhaps, by indirect (fomite) contact followed by self-inoculation of the upper respiratory tract or conjunctival mucosa. The relative importance of these routes is still debated [2]. Receptors for which human influenza viruses have a preference are long glycans terminating in sialic acids linked to galactose by an alpha-2,6 linkage [3]. These receptors are expressed on epithelial cells throughout the respiratory tract—nasal mucosa, paranasal sinuses, pharynx, trachea, bronchi, bronchioles, and alveoli—but their abundance varies per site [4]. In the tracheo-bronchial tree, human influenza viruses attach predominantly to ciliated epithelial cells, and attach more abundantly to tracheal and bronchial epithelium than to bronchiolar epithelium (Fig. 1) [5]. In uncomplicated influenza in humans, the cell types in which human influenza virus replicates *in vivo* only has been determined for the nasal mucosa, where both ciliated and non-ciliated cells are infected [6]. However, *ex vivo* human tissue cultures have shown that epithelial cells of nasopharynx, adenoids, tonsil [7], bronchus and pulmonary alveolus [4] are permissive. *In vitro*, primary cell cultures of human tracheal epithelial cells have shown replication in both ciliated and non-ciliated cells [8].

One of the only descriptions of histologic lesions associated with uncomplicated influenza in humans is from a study of tracheal and bronchial biopsies obtained from six young adults between 1 and 7 days after onset of symptoms [9]. They had a diffuse, superficial, necrotizing tracheo-bronchitis, which was progressively more severe further down the tracheo-bronchial tree. Lesions were already visible at 1 day after onset of symptoms. Damage to the respiratory epithelium ranged from vacuolization, edema, and absence of cilia to extensive desquamation of epithelial cells. In the lamina propria, there was prominent edema and hyperemia, and infiltration with primarily lymphocytes and histiocytes. Inflammatory cell infiltration was limited compared to the extent of epithelial damage. From 2 days after the onset of symptoms, epithelial repair was visible in the form of epithelial metaplasia.

The changes to the bronchial epithelium from influenza virus infection are short-lasting. In a study of bronchial biopsies from patients between 1 and 6 weeks after onset of symptoms, the only significant differences between influenza patients and healthy controls were thickened surface epithelium and slight increase in lymphocytic infiltration of the lamina propria, corresponding with epithelial regeneration and bronchial inflammation, respectively [10;11].

The typical signs and symptoms of uncomplicated influenza are both local (nasal obstruction, cough, sore throat) and systemic (headache, fever, chills, anorexia, myalgia) [1]. These signs and symptoms are due both to the damage at the site of virus replication and to the local and systemic release of cytokines and other inflammatory mediators [12;13].

Primary complication: viral pneumonia

The most common complication of influenza is extension of the viral infection distally to the lung, resulting in pneumonia. In contrast to damage to the tracheo-bronchial epithelium in uncomplicated influenza, damage to the alveolar epithelium has severe consequences for the gas exchange function of the respiratory tract. This damage to alveolar epithelium—consisting of type I and type II pneumocytes—is due to a combination of the direct cytolytic effect of viral infection and the indirect effect of host response [14]. Type I pneumocytes prevent leakage

of fluid across the alveolar-capillary barrier, and type II pneumocytes both resorb fluid from the alveolar lumen and produce lung surfactant that is important for reducing alveolar surface tension. Therefore, damage to these cells allows fluid from the alveolar capillaries to flood into the alveolar lumina. This causes severe, and in some cases fatal, respiratory dysfunction [15].

Risk factors for the development of influenza viral pneumonia include lack of previous exposure to influenza virus with related surface glycoproteins, age greater than 65 years, pulmonary disease, cardiovascular disease, and pregnancy [1]. Individuals who have not been previously exposed to an antigenically related influenza virus lack the protection of the lung against viral infection conferred by specific IgG, which reaches the alveolar lining fluid by transudation from the serum [16–18]. Important chronic underlying pulmonary diseases that predispose influenza patients to hospitalization are chronic obstructive pulmonary disease, asthma, and pulmonary fibrosis [19], which involve remodeling of airways or distal lung parenchyma and thus reduce pulmonary defense against infectious pathogens [20]. There are no clear explanations for the increased risk of influenza viral pneumonia from cardiovascular disease or pregnancy. It has been speculated that pulmonary hypertension secondary to cardiovascular disease or from the increased blood volume in pregnancy may predispose the lung to pulmonary oedema when the alveolar septa are damaged by the virus [21].

Based on attachment studies [5], the primary target cells of human influenza virus in the lower respiratory tract are type I pneumocytes and ciliated bronchiolar epithelial cells, although attachment does occur less frequently to non-ciliated bronchiolar epithelial cells, type II pneumocytes, and alveolar macrophages (Fig. 1). This corresponds with *ex vivo* infection of alveolar epithelial cells by human influenza virus [4]. *In vivo* descriptions of the target cells of influenza virus in fatal pneumonia from any of the three influenza pandemics of the last century are very rare. Specific fluorescence was visible in alveolar epithelial cells and alveolar macrophages in lung tissue of two adult women who died with human influenza virus H2N2 pneumonia during or just after the 1957 pandemic [22;23]. Fluorescence-positive interstitial macrophages were detected in the interstitium and alveolar exudate of 7 of 29 lungs from people who died of influenza in Boston during the 1957 pandemic [24].

The pathological changes to the lung from influenza viral pneumonia have been most commonly described during pandemics and have been recently been reviewed [25]. The acute alveolar injury (diffuse alveolar damage) caused by influenza virus infection is similar to that caused by many other agents that are noxious for alveoli. In the early stage, there is necrosis of alveolar epithelium, characterized by denudation of the alveolar septum and the presence of desquamated pneumocytes in the alveolar lumen. These desquamated cells are shrunken and show pyknosis or karyorrhexis and cytoplasmic vacuolation or hypereosinophilia. The alveolar lumina are flooded by edema fluid with variable admixture of fibrin and erythrocytes (intra-alveolar hemorrhage) (Fig. 2A). In some alveolar lumina, there are many alveolar macrophages. Characteristically, alveoli and alveolar ducts are lined by hyaline membranes, consisting of fibrin-rich edema fluid mixed with the cytoplasmic and lipid remnants of necrotic epithelial cells (Fig. 2B). The alveolar septa are widened due to hyperemia of alveolar capillaries, interstitial edema, and leukocyte infiltration, mainly neutrophils as well as a few eosinophils. These leukocytes also may be present in alveolar lumina. Fibrinous thrombi may be present in the capillaries of alveolar septa and alveolar ducts, as well as in small pulmonary blood vessels (Fig. 2C). Possibly as a result of these thrombi, alveolar septa may be necrotic. The late stage of influenza viral pneumonia is characterized by re-epithelization of the alveoli by type II pneumocytes (type II pneumocyte hyperplasia), interstitial fibrosis of alveolar septa, and infiltration by mononuclear leukocytes, predominantly lymphocytes and plasma cells (Fig. 2D).

In addition to the above alveolar changes, the bronchioles show a necrotizing bronchiolitis, characterized by epithelial necrosis, the formation of hyaline membranes, and infiltration by variable numbers of neutrophils. Changes to the trachea and bronchi are similar to those of uncomplicated influenza. Chronic changes of influenza pneumonia may include squamous metaplasia and interstitial fibrosis [25].

Influenza viral pneumonia often occurs together with, or is followed by, bacterial pneumonia. Prior influenza virus infection may predispose the respiratory tract to bacterial infection by different mechanisms and, vice versa, bacterial infection may enhance influenza virus infection [26]. The bacterial infection results in a different type of inflammation than that caused by influenza virus, with a more prominent infiltration of neutrophils and production of pus: suppurative bronchopneumonia (Fig. 3). A recent review of over 8,000 published autopsy case results from the 1918 pandemic found that the majority of deaths (96%) likely resulted from secondary bacterial pneumonia (Morens D.M., Taubenberger, J.K., Fauci, A.S., unpublished data). As in 1918, most deaths in the 1957 pandemic were due to secondary bacterial pneumonia, although negative autopsy lung cultures were more common than in 1918, possibly due to the widespread administration of antibiotics [27;28]. In one study of the 1957 pandemic, 111/148 (75%) of confirmed fatal cases of influenza had bacteriological and histological evidence of a bacterial pneumonia, mainly due to *Staphylococcus aureus* or pneumococci [29]. In the same study, 30/148 (20%) of fatal cases were considered due to influenza viral pneumonia.

Complications outside the respiratory tract

Human influenza virus primarily infects and causes disease in the respiratory tract. However, human influenza virus infection also is associated with disease in other organs, albeit to a lesser extent. Given the recent reports of extra-respiratory disease from highly pathogenic avian influenza H5N1 virus infection (see below), it is important to revisit these complications of human influenza virus infection.

In general, there are two explanations for the pathogenesis of influenza-associated extra-respiratory complications. The first is that influenza virus spreads via blood to these tissues and replicates there. A likely route for influenza virus to reach blood is by crossing the alveolar-capillary barrier damaged by influenza viral pneumonia. It remains controversial whether viremia routinely occurs during pandemic or seasonal influenza infection. As recently reviewed [30], viremia has been previously reported in influenza virus infection of humans [31–35]. However, several other studies [36–38] failed to detect viremia after onset of illness, suggesting that influenza viremia is rare after onset of symptoms and, if it occurs, is not sustained for long periods [30].

Evidence for replication of influenza virus in extra-respiratory tissues usually comes from detection of virus in these tissues by virus isolation or fully-nested RT-PCR. However, these methods do not exclude the possibility that detected virus originated from blood. The only proof is *in situ* detection of virus by direct immunofluorescence, immunohistochemistry, or *in situ* hybridisation in the tissue concerned. Such reports are rare (e.g., brain: [39;40]; heart: [41]) and further confirmation of the ability of human influenza virus to replicate in extra-respiratory human tissues *in vivo* is badly needed.

The second explanation for the pathogenesis of influenza-associated extra-respiratory complications is suggested by the link between acute respiratory distress syndrome (ARDS) and multi-organ dysfunction syndrome (MODS). ARDS, which may be caused by a variety of insults to the lungs, including influenza virus infection, commonly progresses to MODS [42]. The hepatic, renal, central nervous, gastrointestinal, hematologic, and cardiac systems are most commonly affected [43]. The pathogenesis of MODS has not been elucidated, but is thought

to involve the microcirculation and mitochondrial metabolism. Mechanisms may include the release of cytokines into the circulation [44].

Central nervous system disease

An important complication of influenza A virus infection is central nervous system (CNS) dysfunction, that can take a number of forms [45], including influenza-associated acute encephalopathy (IAAE). This is an uncommon neurological syndrome generally of children and adolescents that typically presents during the early phase of influenza virus infection [45].

There are several hypotheses regarding pathogenesis of IAAE. The most straightforward one is that it is caused by viral infection of the CNS. In support of this hypothesis, influenza virus has been detected occasionally by virus isolation or nested RT-PCR in CSF of patients [46–50] and in brain tissue from fatal cases [39;51]. Virus has been detected in neuropil and ependyma of the brain by direct immunofluorescence [39] and in Purkinje cells of the cerebellum and neurons of pontine nuclei by immunohistochemistry [40]. However, the frequent failure to detect virus in CSF and brain of affected patients despite thorough attempts, as well as the lack of inflammation in brain tissue of fatal cases, suggest that virus infection is, at most, only one of the possible pathogeneses. A second hypothesis for the pathogenesis of IAAE is hypercytokinemia, which does not require extra-respiratory virus infection. The severity of CNS dysfunction is correlated with the concentration of pro-inflammatory cytokines in blood and cerebrospinal fluid [45]. However, some patients with severe influenza-associated acute encephalopathy do not have elevated cytokine levels [47]. A third hypothesis that has been proposed is renal and hepatic dysfunction from influenza virus infection, although it is unclear how this occurs [49].

Grossly, the brain in patients with IAAE shows diffuse swelling, which may be severe [28]. Histologically, this corresponds with severe diffuse cerebral congestion and edema, with the notable absence of inflammatory cell infiltrate [28;39;48]. Vascular changes such as hyalinization of the blood vessel wall and thrombosis may be present [50]. The clinical consequences of these lesions include altered consciousness and convulsions. The outcome is highly variable but may result in persistent neurological sequelae or death [45].

Other rare CNS complications of influenza include post-influenza encephalopathy, Reye's syndrome, Klein-Levin syndrome, post-encephalitic Parkinson's disease, and encephalitis lethargica [45;52;53]. These are not further discussed here.

Myocarditis

Myocarditis has been observed in association with fatal influenza in each of the three pandemics of the previous century (e.g., [28;54;55]), and in interpandemic periods (e.g., [56;57]) but its pathogenesis is poorly understood. The advent of endomyocardial biopsies at the time of acute disease together with sensitive (*in situ*) RT-PCR techniques have made it possible to detect the presence of influenza viral RNA in inflamed myocardial tissue in some cases [41;58] but not in others [59;60;60]. It is not clear what the target cells of influenza virus in human heart tissue are: Cioc and Nuovo [41] detected influenza viral RNA in lymphocytes and macrophages within the myocardium of a person who died suddenly and unexpectedly with marked diffuse myocarditis and marked cardiomyocyte necrosis. Ray et al. [56] detected influenza viral antigen throughout the myocardium (cell types showing antigen expression not stated) of a patient with massive myocardial necrosis and associated lymphocytic and mononuclear infiltrates. The necrosis and inflammatory process in the myocardium could be explained by a combination of direct cytolytic effect of viral infection and the host immune response.

The myocarditis consists of cardiomyocyte necrosis associated with variable infiltration of predominantly mononuclear inflammatory cells. There may be interstitial hemorrhage and edema [28;41;54;61;62]. The clinical outcome differs dependent on the duration of the myocardial disease. If the patient dies acutely of fulminant influenza, the main lesion is in the lungs. If the patient dies later, it may be from heart failure. If the patient survives, the resulting myocardial fibrosis may result in heart block due to problems with electrical conduction [60; 63].

Myositis or myopathy

Myositis or myopathy is sporadically reported as a complication of both influenza A virus and influenza B virus infections [64]. Myopathy is a better term than myositis, because the majority of muscle biopsies from such cases do not show infiltration by inflammatory cells [64]. The pathogenesis of influenza-associated myopathy is poorly understood. The first hypothesis is direct viral invasion of the muscle. This is supported by the isolation of influenza A virus from muscle biopsies of two patients with influenza A virus infection. However, they were unusual cases. One was a 4-year-old boy with Reye's syndrome [65], the other was a 72-year-old man with muscle weakness [66]. Also, direct infection of myocytes has not been proven by immunohistochemistry. The second hypothesis is an immune-mediated process. However, the absence of inflammatory cell infiltrates in the majority of muscle biopsies argue against this [64].

Histologic examination of affected muscle biopsies shows muscle degeneration, necrosis, and regeneration, in some cases associated with inflammatory cells [65–69]. The main clinical symptom of influenza-associated myopathy is transient muscle pain in the lower extremities. Most cases resolve completely. Rarely, severe muscle damage develops that results in myoglobinuria and acute renal failure [64].

Differences between pandemic and interpandemic influenza

Influenza pandemics cause higher morbidity and mortality rates than seasonal epidemics during interpandemic periods. This is mainly due the lack of specific immunity to the new virus, so that infection is more likely to result in complicated disease, in particular pneumonia [1]. This raises the question whether the character of the lesions caused by a pandemic virus are qualitatively different from those caused by an interpandemic virus. Unfortunately, it is difficult to compare the pathology of pandemic and interpandemic influenza, because the vast majority of pathological reports are from pandemic periods, and because pathological reports typically describe the late stage of disease and may be complicated by the effects of therapeutic intervention, so that subtle differences may be masked.

Taubenberger and Morens [25] reviewed the pathology of influenza viral pneumonia in interpandemic periods. The observed lesions were similar to those found during pandemic periods. An interesting observation comes from two studies during an interpandemic period comprising a total of 55 fatal influenza virus infection [70;71]. In these studies, influenza viral antigen was detected in tracheal, bronchial, and bronchiolar epithelial cells, but not in alveolar epithelial cells or alveolar macrophages, even in cases showing diffuse alveolar damage. This contrasts with the findings from the 1957 pandemic [22;23], where viral antigen was detected in alveolar epithelial cells (probably both type I and type II pneumocytes) and alveolar macrophages.

Extra-respiratory complications of influenza described during pandemics, including encephalopathy (reviewed in [52] and [45]), myocarditis (e.g., [56]), and myopathy (reviewed in [64]) also have been reported in interpandemic periods. Based on the available information, the character of these complications does not appear to differ in pandemic and interpandemic

periods. Together, these studies indicate that, although the proportion of infected people who develop complicated influenza is lower during interpandemic periods, the same types of complications occur and are similar in character to those in pandemic periods.

Special features of human infection with avian influenza viruses

Until 1997, direct human infection with avian influenza viruses was considered to be rare and of little consequence to human health. Highly pathogenic avian influenza (HPAI) virus had been isolated from the blood of a man with clinical symptoms of infectious hepatitis ([72;73], and there had been rare reports of transient conjunctivitis from avian influenza virus infection [74;75]. In 1997, this changed when infection with HPAI virus of the subtype H5N1 was diagnosed in people in Hong Kong, resulting in 6 deaths out of 18 confirmed infections despite intensive care [76–78]. Subsequently, one person died of HPAI virus infection of the subtype H7N7 [79], and a low pathogenic avian influenza (LPAI) virus of the subtype H9N2 was identified as the cause of respiratory disease—albeit mild—in humans [80]. Furthermore, sequencing and phylogenetic analysis of the reconstructed influenza virus of the subtype H1N1 that caused the 1918 pandemic indicates that its genes were derived from avian-like influenza strains [81]. Together, these findings indicate that transmission of avian influenza virus from birds to humans might be rare, but is by no means impossible and has potential severe disease consequences, both for the individual infected and, if the virus is able to adapt to its new host, for the whole population.

H5N1 virus

HPAI H5N1 virus continues to circulate among poultry in many countries of Asia, Africa, and Europe and occasionally spreads to humans with often fatal consequences. Understanding of the pathology of H5N1 virus infection in humans is critically hampered by the few autopsies performed on people who have died of the infection. A recent review identified only nine full autopsies, including one of a fetus, out of 216 laboratory-confirmed fatal cases at the time of publication [82].

Based on clinical evaluation of infected people, the primary disease is centred on the lungs [83]. However, the pattern of attachment of H5N1 virus differs markedly from that for human influenza virus, with important consequences for subsequent disease [5]. In the tracheo-bronchial tree, attachment of human influenza virus is strongest in the trachea and progressively decreases lower down in the tracheo-bronchial tree. In contrast, H5N1 virus shows the strongest attachment in the distal part of the tracheo-bronchial tree—the bronchioles—with progressively less attachment towards the trachea (Fig. 1). The pattern of viral attachment also is distinct within the alveoli. Whereas human influenza virus has a preference for type I pneumocytes, H5N1 virus preferentially attaches to type II pneumocytes and alveolar macrophages (Fig. 1). It has been hypothesized that infection of these cell types might explain the high pathogenicity of H5N1 virus: type II pneumocytes are important for surfactant production, fluid transport out of the alveolar lumen, and re-epithelialization after damage, while alveolar macrophages are important for phagocytosis of pathogens and regulation of the inflammatory response in the alveoli [5;84]. The preference of H5N1 virus for attachment to type II pneumocytes is corroborated by studies that show that these cells have avian-type receptors for influenza virus and can be infected by H5N1 virus *in vitro* [4] and *in vivo* [82]. This pattern of viral attachment may also explain why the rare autopsies have shown lesions centred on the alveoli and bronchioles, without reported lesions in trachea or bronchi [82].

The respiratory tract lesions of H5N1 avian influenza in humans are consistent with exudative and proliferative phases of diffuse alveolar damage [82] and thus resemble the lesions of pneumonia from human influenza virus infection. Characteristic features include type II pneumocyte hyperplasia, interstitial infiltration of lymphocytes and in some cases neutrophils,

and predominance of macrophages— some showing hemophagocytosis—in alveolar lumina. Additional histologic features include desquamation of epithelial cells into alveolar lumina, hemorrhage, and bronchiolitis. By immunohistochemistry and *in situ* hybridisation, viral antigens and RNA have been found in type II pneumocytes, as well as ciliated and non-ciliated tracheal epithelial cells [82].

The isolation of the virus from the blood of two patients [85;86] and the detection of H5N1 viral RNA by RT-PCR in 9 of 16 patients [87] suggests that viremia can occur at reasonably high levels and for prolonged periods in people with symptomatic H5N1 virus infection [30]. Such viremia would allow H5N1 virus to spread to extra-respiratory tissues. Indeed, pathological investigations provide evidence for the presence of H5N1 virus in multiple extra-respiratory tissues by immunohistochemistry, *in situ* hybridisation, or both, often in association with lesions. The brain, where H5N1 virus has been found in neurons, is edematous without significant histologic lesions, or with demyelination, necrosis, and accumulation of reactive histiocytes. The intestine, where H5N1 virus has been found in intestinal epithelial cells and in mononuclear cells in the mucosa, has no abnormalities except lymphocytic apoptosis. The liver, where H5N1 virus has been found in Kupffer cells, shows hepatic necrosis, hepatic lipidosis, cholestasis, and Kupffer cell activation. Lymph nodes, where H5N1 virus has been found in lymphocytes, have reactive histiocytes with hemophagocytotic activity. Such evidence of hemophagocytosis also is present in spleen, bone marrow, lungs, and liver. The placenta, where H5N1 virus has been found in Hofbauer cells (fetal macrophages) and cytotrophoblasts, has syncytiocytotrophoblast necrosis, necrotizing deciduitis, and diffuse villitis. The fetus, where H5N1 virus has been found in lung tissue, shows no specific histologic lesions except edema and scant neutrophil infiltration in the lung. The kidney has acute tubular necrosis in absence of the presence of H5N1 virus [82].

The clinical consequences of these lesions typically manifest as severe pneumonia that often progresses rapidly to acute respiratory distress syndrome. Clinical features outside the respiratory tract include vomiting, diarrhea, myalgia, and—rarely— seizures. Nonspecific clinical presentation or atypical presentation (e.g., encephalopathy and gastroenteritis) often result in initial misdiagnosis of subsequently confirmed cases [83;88].

Together, these studies indicate that the primary lesion in fatal cases of both H5N1 virus infection and human influenza virus infection is the same, namely diffuse alveolar damage. The main difference in respiratory disease is the absence of reports of uncomplicated tracheo-bronchitis in H5N1 virus infection, which is the most common manifestation of human influenza virus infection. This may be due to differences in the attachment preferences—upper respiratory tract for human influenza virus, lower respiratory tract for H5N1 virus—or due to incomplete reporting of less severe H5N1 virus infections.

The level and duration of viremia and the extent of extra-respiratory spread appear to be greater for infections with H5N1 virus than with human influenza virus. It is not clear whether this difference is real or an artifact of more detailed pathologic examination with more up-to-date methods of the few H5N1 influenza cases studied.

Other avian influenza viruses (H7N7, H7N3, H7N2, and H9N2)

Between 1959 and 1996, infections with either high or low pathogenic forms of avian influenza virus (H7N7) infection were reported in six people ([72;74;75;89]. The presumed routes of infection were direct exposure to highly pathogenic avian influenza in poultry [72], accidental laboratory infection [89], pre-and post-mortem examination of infected seals [74], and a piece of straw entering the eye while cleaning out a duck house [75]. In five of six cases, a conjunctivitis developed at 1 to 3 days after inoculation and resolved after 4 days to 2 weeks [74;75;89]. Additionally, one person developed an asymptomatic intraepithelial keratitis one

week after inoculation that resolved over the next three weeks [89]. In one of six cases, the virus was isolated from the blood of the patient one month after presumed exposure. The patient had clinical symptoms of an infectious hepatitis, including yellow sclera, dark urine, and loss of appetite. The relationship between these symptoms and isolation of the virus were not clear [72].

In 2003, an outbreak of HPAI H7N7 virus infection in poultry occurred in the Netherlands, and the virus was detected in 86 people who handled affected poultry and three of their family members. The majority of these people (78/89, 88%) presented with conjunctivitis alone, while a smaller proportion had conjunctivitis and influenza-like illness (5/89, 6%) or influenza-like illness alone (2/89, 2%). Six of seven cases of influenza-like illness were mild. However, one patient developed severe pneumonia and died from acute respiratory distress syndrome and related complications. On autopsy, significant pathological changes were limited to the respiratory tract. Grossly, the lungs were edematous, emphysematous, firm, and about three times the normal weight. Histologically, there was severe diffuse alveolar damage, characterized by flooding of the alveolar lumina with serosanguineous fluid mixed with fibrin and neutrophils (Fig. 4). Although the virus was isolated from postmortem lung samples, viral antigen could not be detected in lung tissue by immunohistochemistry [79;90].

In 2004, an outbreak of HPAI H7N3 virus infection in poultry occurred in Canada. Two people who had direct conjunctival exposure to infected poultry were infected and developed conjunctivitis and mild influenza-like illness. Disease developed one to 3 days after inoculation and resolved fully [91]. In 2006, one person who was exposed to infected poultry from a U.K. farm with a LPAI H7N3 virus outbreak became infected and developed conjunctivitis [92].

Between 1999 and 2003, at least four separate human cases of LPAI H9N2 virus infection have been confirmed in China [80;93]. One of these cases had a history of probable contact with live chickens before illness; the others had no history of contact with animals. All four were children between 1 and 5 years of age and presented with influenza-like illness. In two children, symptoms included fever, anorexia, inflamed pharynx, and vomiting. In the other two, they included fever and cough. Three of four children recovered after two to six days, the outcome for the last child was not stated.

Influenza hemagglutinin receptor binding preferences for either alpha-2,3 or alpha-2,6 receptors clearly play a role in host-virus interaction but changes in receptor specificity alone are not adequate to account for host adaptation and transmissibility [4;94–96]. Infections with avian influenza viruses of H7 subtype have been associated predominantly with conjunctivitis, even though most H7 and H5 viruses share a predominant alpha-2,3 receptor specificity. Thus, other factors must account for the conjunctival tropism of H7 influenza viruses. Some of the human infections with H9N2 viruses were associated with increased specificity for alpha-2,6 receptors prevalent in human upper respiratory tract [4;97].

Perspectives

Influenza remains a major public health concern, both for its pandemic potential and for the impact of seasonal influenza. Furthermore, direct bird-to-human transmission of avian influenza viruses, particularly of the H5 and H7 subtypes, have caused human disease and mortality. There are many gaps in our knowledge of the pathogenesis and pathology of influenza in humans, despite published pathology studies of influenza virus infection going back at least to 1889 [25]. Because the majority of these studies by necessity took place at the time of pandemics, the last of which occurred in 1968, they lacked the benefit of advanced immunological and molecular biological techniques at our disposal today. This precluded accuracy in both localization of virus in tissues and identification of cell types involved.

Therefore, directed pathology studies, based both on biopsies of influenza patients and autopsies of fatal cases, need to be performed to fill in these gaps. These studies ideally should cover the broad scale of presentation of both human and avian influenza virus infections in humans, from uncomplicated disease to pneumonia and extra-respiratory complications. Also, these pathology studies need to be integrated with virological, immunological, and clinical aspects of influenza virus infection. The knowledge gained can be used to compare and contrast human and avian influenza virus infections in humans. It can also supplement knowledge from laboratory, clinical, and population studies to gain a better overall picture of influenza in humans, in order to guide strategies to combat this many-faceted disease.

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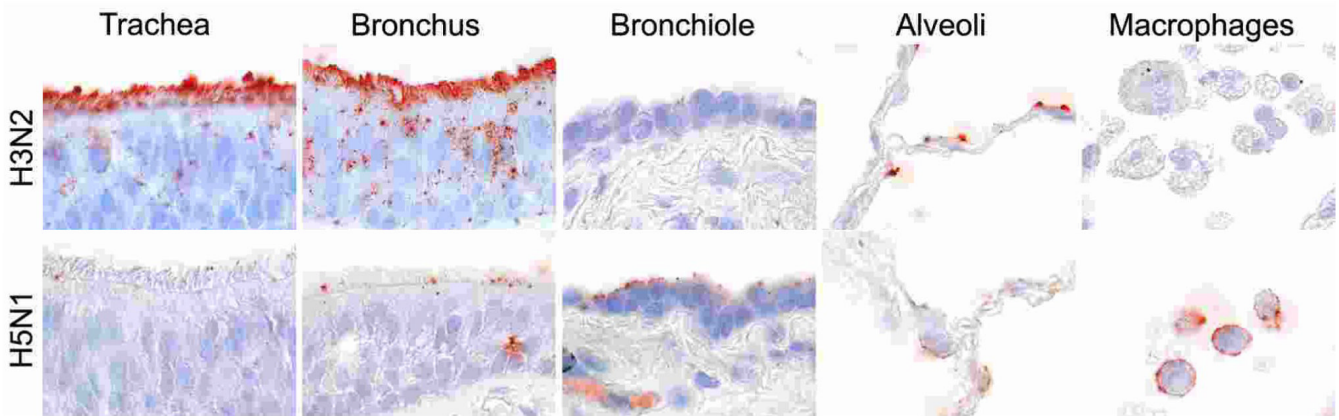

Figure 1.
Attachment of human H3N2 influenza virus (top row) and highly pathogenic avian H5N1 virus (bottom row) in human trachea, lower respiratory tract (bronchus, bronchiole, and alveoli), and alveolar macrophages [5].

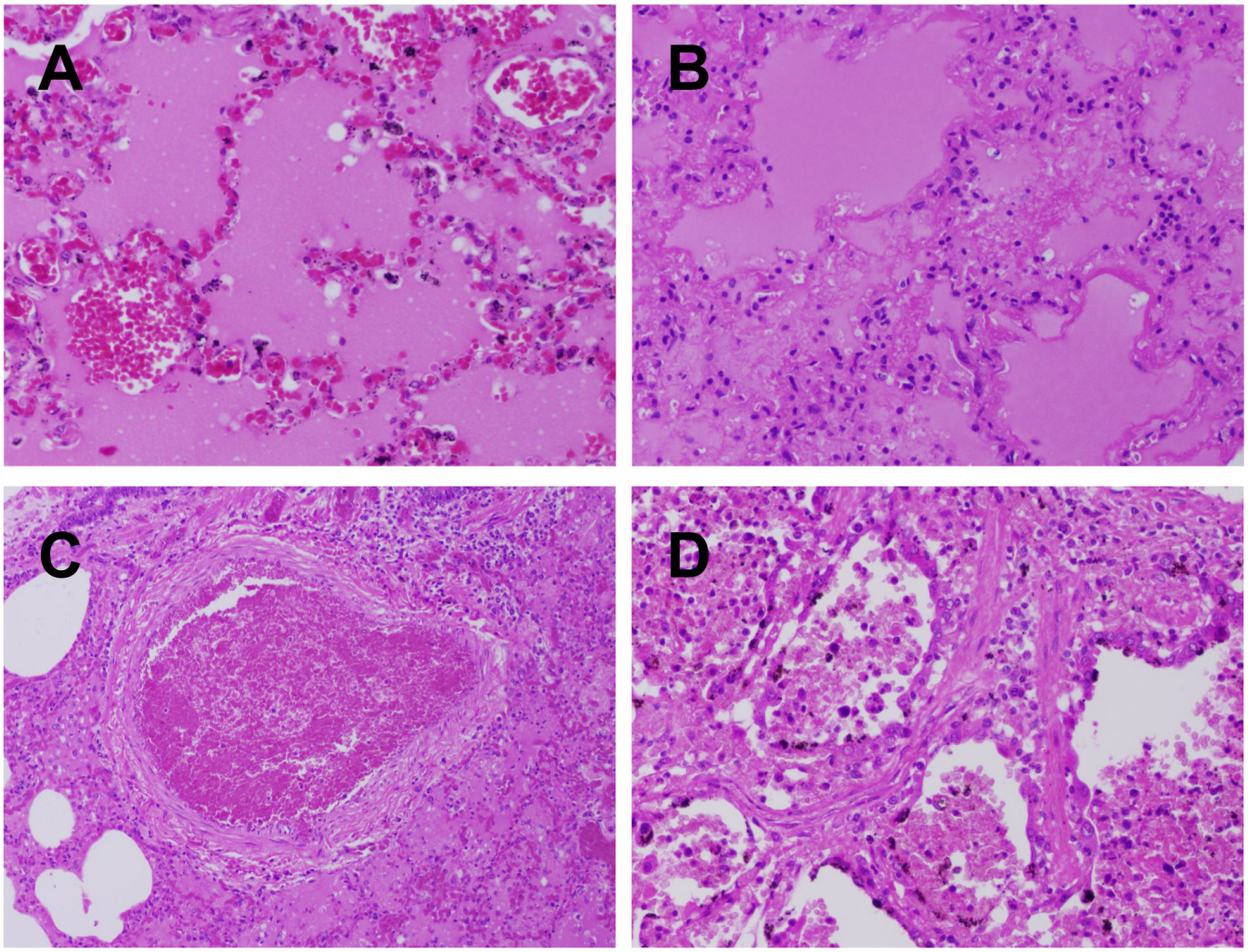

Figure 2.
Charastic lesions of human influenza virus infection in the lung. (A) Acute massive alveolar edema and congestion (1957 pandemic autopsy case, original magnification 200X). (B) Acute massive alveolar edema with hyaline membrane formation and interstitial inflammation (1918 pandemic autopsy case, original magnification 200X). (C) Thrombus in a small pulmonary vessel (1918 pandemic autopsy case (original magnification 40X). (D) Regeneration as evidenced by alveolar type II pneumocyte hyperplasia and interstitial fibrosis (1918 pandemic autopsy case, original magnification 200X).

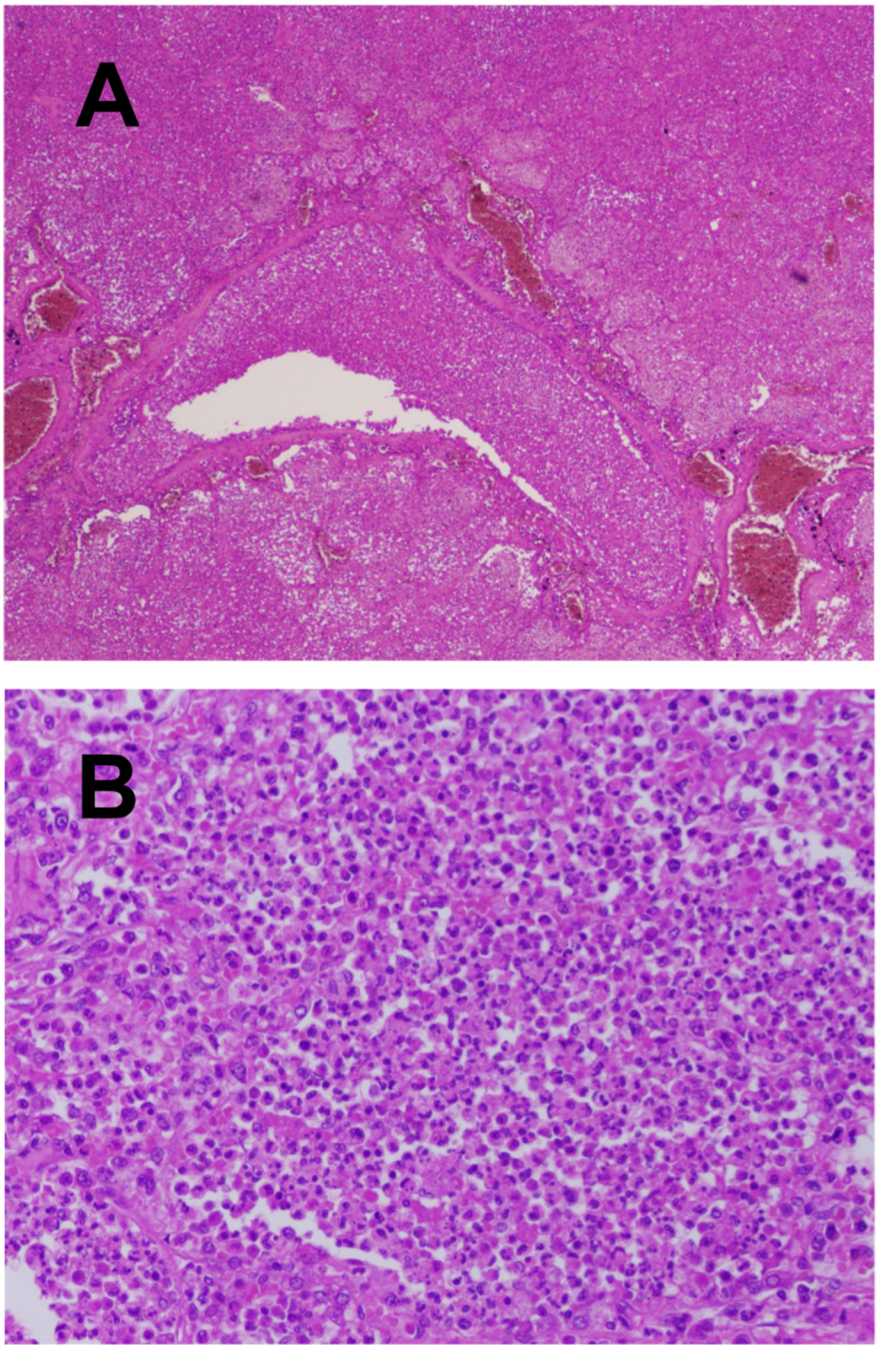

Figure 3.
Lesions of secondary bacterial infection in fatal human influenza cases. (A) Secondary bacterial bronchopneumonia with neutrophils in the lumen of a bronchiole with transmural infiltration of wall and into surrounding lung tissue (1918 pandemic autopsy case, original magnification 40X). (B) Secondary bacterial bronchopneumonia with neutrophils filling the lumen of an alveolus (1918 pandemic autopsy case, original magnification 40X).

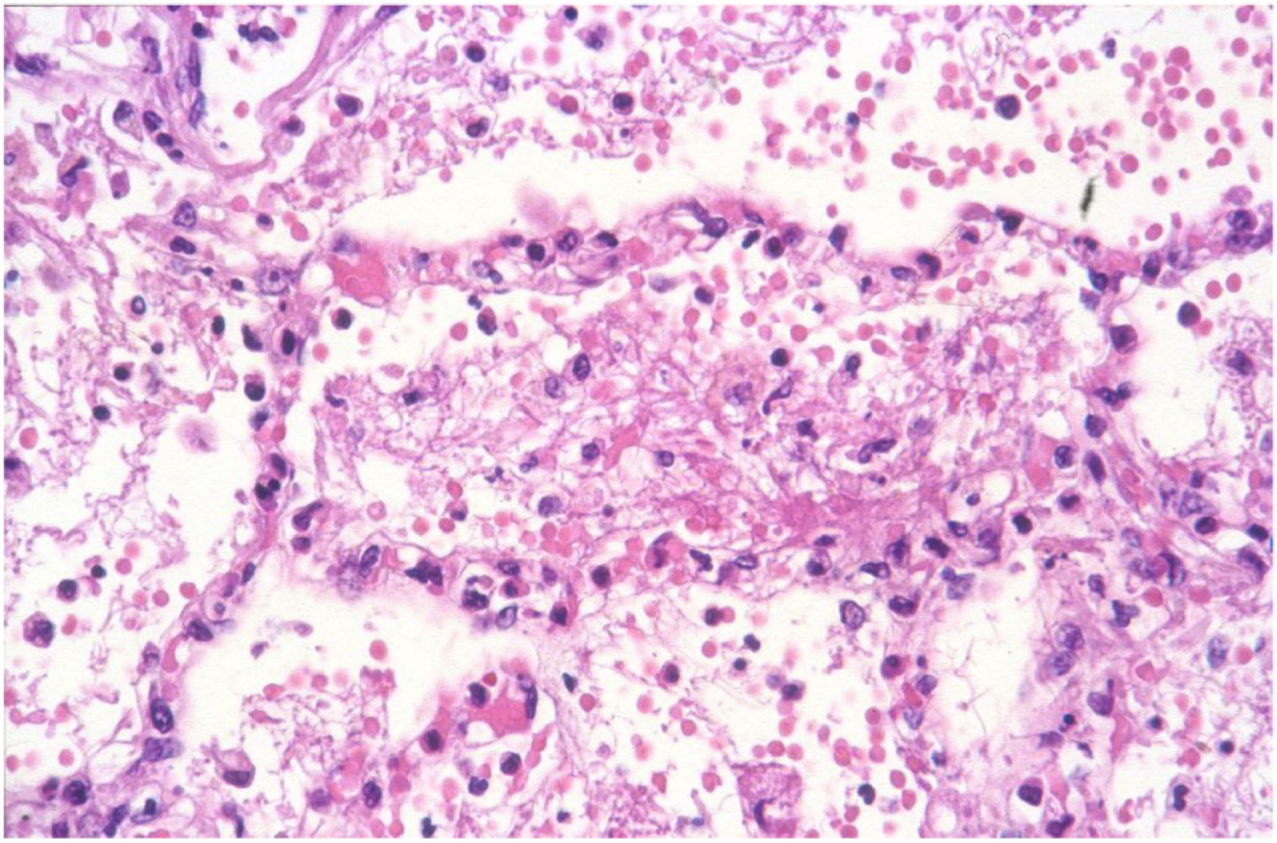

Figure 4.
Lesions of highly pathogenic avian influenza H7N7 virus infection in the lung [79]. There is diffuse alveolar damage, with serosanguineous fluid mixed with fibrin and neutrophils in alveolar lumina.

パンデミック（H1N1）2009 ウイルスに対する
献血適合性、血液製剤の安全性、血液供給の維持の評価のためのガイダンス案

2009 年 11 月　ガイダンス草案（この文書は意見聴取のみを目的としたものである。）

1　導入

●この文書は、パンデミック（H1N1）2009 ウイルスに対して、献血適合性と血液製剤の安全性を評価し、また、血液と血液製剤の供給量を維持するために、勧告を行うものである。

2　背景

●２００９Ｈ１Ｎ１インフルエンザウイルスによるウイルス血症については、限られた情報しか得られていないが、米国その他の地域において、輸血により季節性インフルエンザに感染した事例は報告されておらず、同様に輸血により２００９Ｈ１Ｎ１インフルエンザに感染した事例は報告されていない。

●現時点において、２００９Ｈ１Ｎ１インフルエンザに感染した無症候状態の者の血液や血清から２００９Ｈ１Ｎ１インフルエンザウイルスは分離されていないが、研究は継続中である。

●輸血による２００９Ｈ１Ｎ１インフルエンザ感染の可能性は不明のままである。

3　勧告

献血の延期

●　現時点で利用可能なデータに基づけば、２００９Ｈ１Ｎ１インフルエンザに感染した者、又は感染の疑いのある者、若しくはインフルエンザ様症状を呈している者と接触した者に対して献血を制限する理由はない。

●　２００９Ｈ１Ｎ１インフルエンザに感染した者又は感染の疑いのある者は、献血の日に健康状態が良好であることを確保するため、解熱剤なしで熱が下がり、症状がなくなってから、少なくとも２４時間経過するまでは献血を制限すべきである。

●更に、現時点で利用可能なデータに基づけば、２００９Ｈ１Ｎ１インフルエンザワクチン（生ワクチン又は不活化ワクチン）を接種した者やオセルタミビル（商品名タミフル）及びザナミビル（商品名リレンザ）の予防投与を受けた者について、献血を制限する理由はない。

製品管理

●　献血後４８時間以内に供血者が２００９Ｈ１Ｎ１インフルエンザに感染、又は感染の疑いがある、若しくはインフルエンザ様症状を呈したという情報が寄せられた場合、メディカル･ディレクターは、既存の標準作業手引書（SOP）に基づいて、当該献血血液の安全性について評価しなければならない。

Guidance for Industry

Recommendations for the Assessment of Blood Donor Suitability, Blood Product Safety, and Preservation of the Blood Supply in Response to Pandemic (H1N1) 2009 Virus

DRAFT GUIDANCE

This guidance document is for comment purposes only.

Submit comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. Submit electronic comments to http://www.regulations.gov. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or email ocod@fda.hhs.gov, or from the Internet at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm..

For questions on the content of this guidance contact OCOD at the phone numbers listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
November 2009

Table of Contents

Contains Nonbinding Recommendations

Draft – Not for Implementation

Guidance for Industry

Recommendations for the Assessment of Blood Donor Suitability, Blood Product Safety, and Preservation of the Blood Supply in Response to Pandemic (H1N1) 2009 Virus

This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

This guidance document provides recommendations for assessing blood donor suitability and blood product safety and maintaining blood and blood product availability in response to pandemic (H1N1) 2009 virus. It is intended for establishments that manufacture Whole Blood and blood components intended for use in transfusion and blood components intended for further manufacture, including recovered plasma, Source Plasma and Source Leukocytes. Within this guidance, "you" refers to blood establishments; "we" refers to FDA.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidance means that something is suggested or recommended, but not required.

II. BACKGROUND

A. Epidemiology and Pathogenesis

The 2009 H1N1 pandemic is caused by a novel influenza A virus of swine origin. On April 26, 2009, then Department of Health and Human Services (DHHS) Acting Secretary Charles E. Johnson, pursuant to section 319 of the Public Health Service Act, 42 U.S.C. § 247d, declared a public health emergency when a novel swine-origin 2009 influenza A (H1N1) virus was identified in California, Texas, Kansas, and New York. The pandemic influenza H1N1 virus has since spread quickly to all fifty states and globally. In June 2009, the World Health Organization (WHO) declared a Phase 6 Level of Pandemic Influenza Alert. This declaration was based upon a standard definition reflecting worldwide spread of the pandemic (H1N1) 2009 virus and the observed

efficiency of human to human transmission. Importantly, a declaration of a pandemic is independent of the severity of illness caused by the virus or the degree of infrastructure disruption. On July 24 2009, DHHS Secretary Kathleen Sebelius renewed DHHS' April 2009 determination that a public health emergency exists nationwide involving pandemic influenza H1N1 that has significant potential to affect national security.

From April 15, 2009 to July 24, 2009, states reported to the Centers for Disease Control and Prevention (CDC) a total of 43,771 confirmed and probable cases of novel influenza A (H1N1) infection. Of these cases reported, 5,011 people were hospitalized and 302 people died.[1,2] From August 30, 2009 to October 24, 2009, 25,985 hospitalizations and 2,916 deaths attributed to influenza and influenza-like illnesses have been reported in the United States (U.S.). CDC has developed a model to estimate the true number of cases in the U.S. The model took the number of cases reported by states and adjusted the figure to account for known sources of underestimation (e.g., not all people with pandemic influenza H1N1 seek medical care, and not all people who seek medical care have specimens collected by their health care providers). Using this approach, it is estimated that more than one million people became infected with novel influenza A (H1N1) between April and June 2009 in the U.S.[3]

The symptoms of human influenza disease caused by pandemic (H1N1) 2009 virus are similar to the symptoms of seasonal flu and include fever, cough, sore throat, runny or stuffy nose, body aches, headache, chills and fatigue. A significant number of people who have been infected with pandemic (H1N1) 2009 virus also have reported diarrhea and vomiting.[4]

The most severe outcomes have been reported among individuals with underlying health problems that are associated with high risk of influenza complications. Pandemic (H1N1) 2009 virus currently remains sensitive to oseltamivir (Tamiflu) and zanamivir (Relenza), though sporadic cases of resistance to oseltamivir have been reported. At this time, there is insufficient information to predict how severe the pandemic (H1N1) 2009 virus outbreak will be in terms of illness and death or infrastructure disruption, or how it will compare with seasonal influenza.

B. Potential Impact of the H1N1 Pandemic on Blood Product Safety and Availability

There is limited information available on pandemic (H1N1) 2009 virus viremia, especially during the asymptomatic period. No case of transfusion transmitted seasonal

[1] http://www.cdc.gov/h1n1flu/update.htm, (Accessed Nov. 2, 2009).
[2] CDC discontinued reporting of confirmed and probable cases of novel H1N1 infection on July 24, 2009. The most recent total numbers of hospitalizations and deaths due to H1N1 are available on the CDC website. http://www.cdc.gov/h1n1flu/update.htm, (Accessed Nov. 2, 2009).
[3] http://www.cdc.gov/h1n1flu/surveillanceqa.htm, (Accessed Nov. 2, 2009).
[4] http://www.cdc.gov/h1n1flu/sick.htm, (Accessed Nov. 2, 2009).

influenza has ever been reported in the U.S. or elsewhere, and, to date, no cases of transfusion transmitted pandemic influenza H1N1 have been reported. At this time, the pandemic (H1N1) 2009 virus has not been isolated from blood or serum of asymptomatic, infected individuals; however, studies are ongoing. Furthermore, the potential for transmission of pandemic influenza H1N1 through blood transfusion remains unknown.

In some previous studies, other Influenza A viruses were isolated from blood, and throat secretions or nasopharyngeal mucosa of children with clinical manifestations of influenza (Refs. 1-2). The virus was isolated from blood and throat washings of 1/29 healthy asymptomatic contacts who became ill 12 hours after the specimens were obtained (Ref. 3). From another study, virus isolation was reported from lungs, adrenals and meninges (from autopsy) which indicated that viremia must have been present (Ref. 4). In humans experimentally infected by nasal inoculation, viremia was observed in 4/15 subjects using sensitive culture methods. Symptoms occurred 2 days after initial viremia and one patient remained asymptomatic throughout the study period (22 days) (Ref. 5). However, other investigators were unable to detect viremia in 27 subjects using a similar virus strain and assay methods (Ref. 6).

The pandemic influenza H1N1 virus is a large lipid-enveloped virus. Validation studies performed by product manufacturers have shown that viruses with similar characteristics to the pandemic influenza H1N1 virus are effectively inactivated and/or removed during manufacturing of plasma derivatives.

Due to its known potential for rapid spread, pandemic (H1N1) 2009 virus has the potential to cause disruptions in the blood supply. A significant number of blood donors, blood establishment staff, and vendors of blood-related supplies (e.g., manufacturers of reagents and blood bags) could be affected as individuals become ill or need to care for ill family members. At the same time, during a widespread outbreak of disease caused by the pandemic (H1N1) 2009 virus, it is anticipated that the demand for blood and blood components may be reduced due to postponement of elective surgery, were that to become necessary in some affected healthcare settings.

In addition, the usual paradigm for ensuring blood availability in response to local disasters (i.e., hurricanes) may not be available under severe pandemic scenarios. In local disasters, interregional transfer of blood from unaffected to affected areas has been an effective strategy. However, in a more severe pandemic scenario, international, national, and regional outbreaks may occur simultaneously and a pandemic wave may last for months. Therefore, advanced planning is reasonable to prepare for the possible need to mitigate the effects of a more severe pandemic and to help ensure that blood is available in affected areas

Standard precautions for avoidance of contact with respiratory secretions may help to reduce the transmission of pandemic (H1N1) 2009 virus in blood and plasma collection establishments. The CDC has issued recommendations for infection control in the

community[5], places of business[6], and in health care settings[7]. CDC also has issued "Interim Infection Control Guidance on 2009 H1N1 Influenza for Personnel at Blood and Plasma Collection Facilities."[8] We recognize the importance of the CDC recommendations for infection control in blood and plasma collection establishments.

III. RECOMMENDATIONS

FDA, in communication with DHHS Office of Public Health and Science, CDC, and the AABB Interorganizational Task Force on Pandemic Influenza and the Blood Supply, monitors blood availability closely. Similarly, we anticipate that you will maintain close communications with your hospital customers to anticipate demand for blood and blood components.

While shortages are not forecast at present, we are reminding you of regulatory pathways and providing regulatory clarification that may be helpful to you both in dealing with the current outbreak and in continuing to stay prepared.

We will continue to review any new scientific information about the potential risk of transfusion transmission of pandemic (H1N1) 2009 virus. We also will monitor closely the impact of the pandemic on blood availability. As our knowledge base grows, we may revise the recommendations in this guidance document as appropriate.

A. Training of Back-Up Personnel

Under 21 CFR 211.25 and 21 CFR 606.20, personnel performing critical functions in blood establishments must be adequate in number, educational background, training and experience, including professional training as necessary, or combination thereof, to assure competent performance of their assigned functions. Given the unknown extent of the disease caused by pandemic (H1N1) 2009 virus, we recommend that you have adequate back-up personnel, in the event of anticipatable personnel shortages. We further recommend that where possible, more than one back-up person should be trained for each critical function. Any such back-up personnel should be trained pursuant to your existing training program. We also recommend that as provided in your training program, you document this training and/or re-training.

[5] http://www.cdc.gov/h1n1flu/guidance/exclusion.htm, (Accessed Nov. 2, 2009).
[6] http://www.cdc.gov/h1n1flu/business/guidance, (Accessed Nov. 2, 2009).
[7] http://www.cdc.gov/h1n1flu/guidelines_infection_control.htm, (Accessed Nov. 2, 2009).
[8] http://www.cdc.gov/h1n1flu/guidance/blood_facilities.htm.

B. Blood Donor Suitability, Donor Deferral and Product Management

Blood Donor Suitability

In general, a donor medical history is obtained at the time of blood collection. However, under 21 CFR 640.3(a) and 21 CFR 640.63(a), the suitability of a donor as a source of Whole Blood or Source Plasma, must be made on the *day of collection* from the donor. These regulations do not explicitly define the term *day of collection.* Occasionally, donor's responses to the donor questions presented before collection are found to be incomplete upon review by the blood establishment. You may clarify a donor's response to the donor history questionnaire or obtain omitted responses to questions within 24 hours of the collection.

Blood Donor Deferral

- Under current FDA regulations, blood donors must be in good health, as indicated in part by normal temperature and free of acute respiratory diseases on the day of collection (21 CFR 640.3(a), (b)(1) and (4) and 21 CFR 640.63(a), (c)(1) and (7)).

- Available data do not currently support donor deferral for exposure to or contact with a person who has confirmed or probable pandemic (H1N1) 2009 influenza or influenza-like symptoms.

- To ensure donors are in good health on the day of donation as required under 21 CFR 640.3(b) and 21 CFR 640.63(c), donors with a confirmed or probable case of pandemic (H1N1) 2009 virus infection should be deferred until at least 24 hours after they are free of fever without the use of fever reducing medications[9] and they are otherwise asymptomatic.

- Available data do not support the deferral of donors following vaccination with live attenuated influenza vaccines (LAIV) or inactivated influenza vaccines against pandemic (H1N1) 2009 virus or for prophylactic use of the antiviral medications oseltamivir (Tamiflu) and zanamivir (Relenza). However, consistent with the recommendation above, donors taking anti-viral medications for confirmed or probable pandemic (HIN1) 2009 virus infection should be deferred until at least 24 hours after they are free of fever without the use of fever reducing medications[10] and they are otherwise asymptomatic.

[9] A daily dose of pediatric aspirin (81 mg) is not considered fever-reducing medication.
[10] A daily dose of pediatric aspirin (81 mg) is not considered fever-reducing medication.

Blood Product Management

The recommendations in this section apply to donations of Whole Blood and blood components intended for transfusion. This section does not apply to blood components intended for further manufacture (recovered plasma, Source Plasma, Source Leukocytes) since validation studies have shown that viruses with similar characteristics to pandemic (H1N1) 2009 virus are effectively inactivated and/or removed during manufacturing of plasma derivatives.

- Upon receipt of post donation information about a donor with confirmed or probable pandemic (H1N1) 2009 disease or influenza like illness within 48 hours after the donation, the Medical Director should evaluate the safety of the previously donated products consistent with existing Standard Operating Procedures (SOPs).

C. Changes to an Approved Application

As provided under 21 CFR 601.12(c)(5), we have determined that the following changes to an approved application for licensed blood establishments may be submitted as a "Supplement-Changes Being Effected".

- Use of a different outside test lab, provided the test lab is registered with FDA and has been performing donor testing.
- Implementation of self-administered donor history questionnaires, provided you follow the critical control points described in FDA's "Guidance for Industry: Streamlining the Donor Interview Process: Recommendations for Self-Administered Questionnaires" (July 2003), and the submission contains the content recommended for all self-administered procedures and computer assisted interactive procedures outlined in the same guidance.

The recommendations set forth above supersede the recommendations in FDA's "Guidance for Industry: Changes to an Approved Application: Biological Products: Human Blood and Blood Components Intended for Transfusion or for Further Manufacture" (July 2001) at section IV.C and FDA's "Guidance for Industry: Streamlining the Donor Interview Process: Recommendations for Self-Administered Questionnaires" (July 2003) at section IV.A, respectively (in both of these guidances, we previously had determined that these changes would require a "Supplement – Changes Being Effected in 30 Days").

IV. BIOLOGIC PRODUCT DEVIATION AND FATALITY REPORTING

Licensed manufacturers, unlicensed registered blood establishments, and transfusion services are subject to reporting requirements with respect to the reporting of product deviations under

21 CFR 606.171. Blood establishments are not expected to submit biological product deviation reports for post-donation information related to pandemic (H1N1) 2009 virus. If a complication of blood transfusion results in the fatality of a recipient, blood establishments must report the fatality to FDA as soon as possible (21 CFR 606.170(b)).

V. COLLECTION AND USE OF CONVALESCENT PLASMA

Plasma obtained after recovery from an acute infection (convalescent plasma) generally contains highly-specific antibodies directed at the infectious agent, and has theoretical potential to serve as a therapeutic product. In consideration that circumstances could arise where vaccines and antiviral drugs might not be sufficiently available, or where a patient is not responding to approved therapies, transfusion of convalescent plasma has been discussed as a possible empirical treatment during an influenza pandemic. (Ref. 7-8)

In July 2009, the WHO Blood Regulators Network issued a position paper[11] on the collection and use of convalescent plasma as an element in pandemic influenza planning. This paper recommends that scientific studies on the feasibility and medical effectiveness of the collection and use of convalescent plasma, and possibly fractionated immunoglobulins, should be explored through clinical trials. FDA encourages the development of new, safe and effective therapies for influenza. Because of its experimental nature, collection and administration of convalescent plasma should be conducted only under an Investigational New Drug Application. Blood establishments that intend to manufacture convalescent plasma should contact FDA to discuss their plans.

VI. IMPLEMENTATION

This guidance has been issued for comment purposes only.

[11] http://www.who.int/bloodproducts/brn/BRNPosition-ConvPlasma10July09.pdf, (Accessed Nov. 2, 2009).

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