

Preparing for the next flu pandemic: from SARS to avian flu

Zhong N

There were three episodes of severe acute respiratory syndrome (SARS) (including one pandemic) in China from 2003 to 2004. Starting from 2002, we are also aware of the increasing alert of an avian flu pandemic. Epidemics of SARS and avian flu have posed huge threats to social stability, economic prosperity and human health in this country. Our experience during the SARS outbreaks can be of great advantage in protecting people against SARS resurgence, and potential attacks of highly pathogenic avian influenza viruses.

ANIMAL SOURCE CONTAINMENT

Despite arguments from genomic evolution analysis that SARS-coronavirus (SARS-CoV) was transmitted to small carnivores after the epidemic of SARS in humans,⁽¹⁾ it has been clearly shown that the SARS-CoV-like virus in bats jumped into some unknown mammal and changed significantly before infecting the market civets, where the viruses amplified in burden, mutated with genomic deletions, and jumped into humans, based on the following epidemiological study:⁽²⁾

- (1) The initial cases of SARS were chefs and animal handlers of wild game.
- (2) The SARS-CoV can only be found in the market civets (26% of civets in Guangdong) and wild horseshoe bats, but not in farmed or wild civets.
- (3) The genetic mutation as demonstrated by the Ka/Ks ratio is very high for the market civet virus and human virus, which means that both are not natural reservoirs for the SARS-CoV. The bat SARS virus is only 90% identical with the civet or human SARS-CoV.
- (4) The SARS-CoV in civets has a 29-bp sequence that is not present in the human virus.

In this regard, wildlife marketing represents a dangerous source of possible new infections among animals, which may undermine the prevention of SARS. The same is true with avian flu. Currently, the Chinese government has set up strict regulations against wildlife marketing, including bans on rearing, trading, transporting and slaughtering wild animals for dietary purposes. Successful prevention of the second SARS event from escalating into another epidemic in China convincingly proved the pivotal role of governmental policy in controlling the wildlife market. As illegal wildlife marketing has not yet diminished completely in southern China, rigorous intervention will continue to be in force. With regard to avian flu infection from poultry to human beings, all chickens (or ducks) should

be killed on the farm or poultry market where an outbreak is recognised, with all domestic fowl culled, and all close contacts quarantined within an area of 3 km in diameter; all illegal poultry markets should be banned.

PATHOGENESIS

Lung damage caused by SARS-CoV and avian flu is characterised as diffuse alveolar damage (DAD), which is different from neutrophil infiltration as found in classic pneumonia. In addition to DAD, SARS-CoV (avian flu virus, in particular) infections are prone to cause multi-organ dysfunction, which in turn leads to a high death rate. Immunopathological injury of host cells triggered by the immune response to virus plays a key role in the pathogenesis of virus infections. During the interactions among the virus, macrophages, monocytes and airway epithelium, many cytokine/chemokines released from activated immune cells not only take part in the process of antiviral immune response, but are also involved in cell damage and development of organ dysfunction.⁽³⁻⁵⁾

In a study of the expression profiles of 14 cytokines/chemokines in blood samples from SARS patients, Jiang et al had found that interferon-inducible protein-10 (IP-10) was markedly elevated during the early stages of SARS, and remained at a high level until convalescence.⁽⁶⁾ Xu et al found that interferon-gamma-induced monokine (Mig) was expressed in gliocytes in the brain mesenchyma of SARS patients.⁽⁷⁾ IP-10 has been shown to be a potent chemoattractant for activated T cells, natural killer cells and monocytes after viral infection; elevated IP-10 may attract these cells, leading to acute immune inflammation.^(8,9) The IP-10 neutralising antibody can effectively inhibit spike-protein-induced IP-10 activity, thereby attenuating damage of endothelial cells of the umbilical cord (Yong XY, personal data, 2008).

Similarly, H5N1 virus-induced cytokine release cascade may contribute to disease severity.^(10,11) In a study of 18 individuals infected with H5N1, de Jong et al found low peripheral blood T-lymphocyte counts and high chemokine and cytokine levels (IP-10, Mig and MCP-1 in particular) in H5N1-infected individuals, particularly in those who died, which correlated with pharyngeal viral loads, suggesting that high viral load and the resulting intense inflammatory responses were central to influenza pathogenesis.⁽¹²⁾ Efforts should be focused not only on preventing such intense cytokine response by early diagnosis and effective antiviral treatment, but also on modulating (or inhibiting) cell damage caused by a cytokine release cascade.

Guangzhou Institute of Respiratory Diseases, First Affiliated Hospital of Guangzhou Medical College, 151 Yanjiang Rd, Guangzhou 510120, China

Zhong N, FRCSE
Professor of Medicine

Correspondence to:
Prof Nanshan Zhong
Tel: (86) 20 8332 8322
Fax: (86) 20 8335 0363
Email: nanshan@vip.163.com

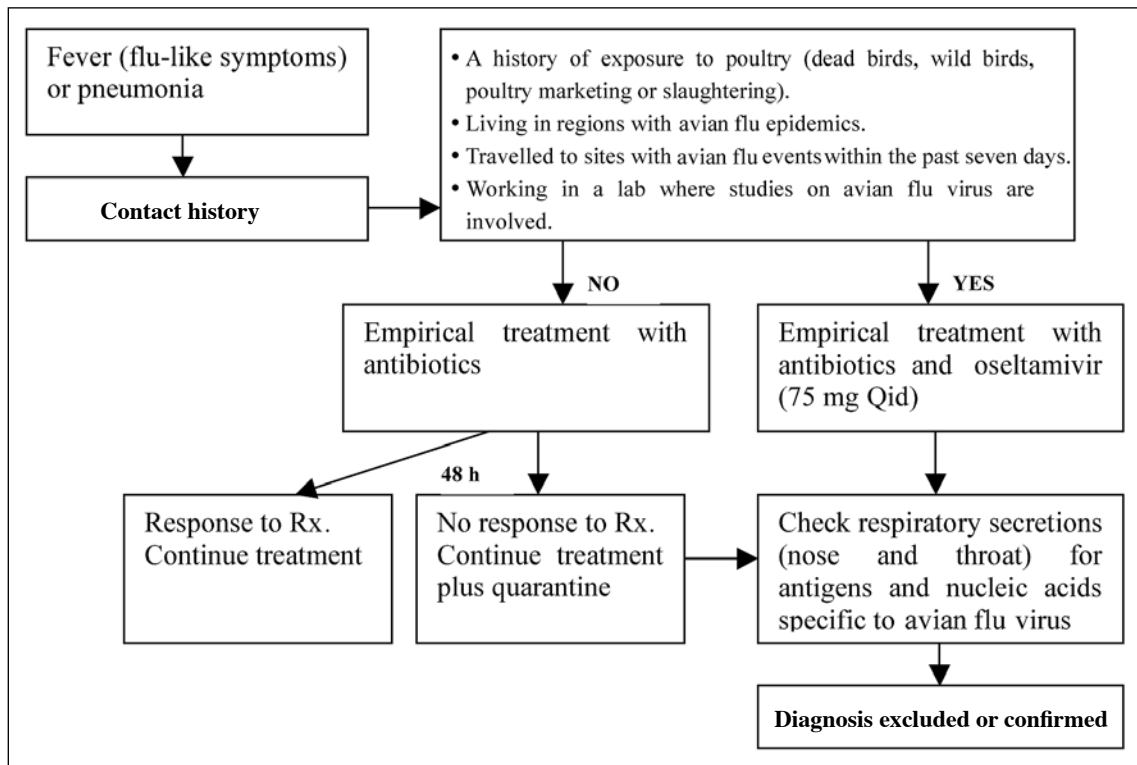


Fig. 1 Algorithm for early diagnosis of avian influenza in China (2008).

EARLY DETECTION

Early identification of an index patient has been the key to initiating preventive measures against the spreading of SARS. Since 2004, we have been setting up a monitoring system for patients with pneumonia. Those who presented with fever, pneumonia and normal or low white cell counts routinely receive serum RT-PCR and IgG testing of SARS-CoV.⁽¹³⁾ During the 2002/2003 SARS outbreak, a confirmed diagnosis of SARS was made based on symptoms, contact history and a retrospectively positive SARS-CoV IgG testing (which was not clinically relevant until 10–14 days after onset of symptoms). Early diagnosis of SARS using RT-PCR to detect viral RNA was available, but the sensitivity and specificity of this technique was not promising.⁽¹⁴⁾ Ho et al have reported on regular nasopharyngeal swab screening with nested RT-PCR analysis. 25 out of 217 (11.5%) well-protected healthcare workers (HCWs) who had been exposed to SARS patients showed viral colonisation without demonstration of SARS-CoV specific antibodies.⁽¹⁴⁾ This screening in conjunction with a daily recording of body temperature in all first-line HCWs may provide an effective way for early protection. A simpler method to detect nucleocapsid phosphoprotein (N-protein), developed by Che et al, subsequently became available,⁽¹⁵⁾ and has been demonstrated to be a highly sensitive test and specific marker in diagnosing SARS.

Similarly, detection of viral RNA by means of conventional or real-time RT-PCR remains the best method for the initial diagnosis of H5N1 infection.⁽¹⁶⁾ These assays can provide results within 4–6 hours, and can be performed under biosafety level two settings. Apart from SARS-CoV, diagnostic yields are higher with throat specimens than with nasal swabs, because

of higher viral loads of influenza A (H5N1) in the throat.⁽¹²⁾ Again, the detection of anti-H5 antibodies (2–3 weeks after infection) is essential for epidemiological investigations and may provide retrospective diagnostic confirmation in patients.⁽¹⁶⁾ Recently, an early diagnosis flow chart (Fig. 1) has been set up in China, which will be made available in all medium and large cities, and a number of small cities.⁽¹⁷⁾ In addition to monitoring body temperature using infrared acquisition, an earlier screening of nasopharyngeal inflammation was developed using thermographic techniques (Yu MS, personal data), which may be more useful to recognise individuals with early avian flu infection, as de Jong had mentioned that the avian flu virus was easier to detect in throat swabs than in nasal swabs.⁽¹⁸⁾

RAPID INFORMATION NETWORK FOR SARS

Since 2003, China has enforced her legislation on the surveillance, report and pre-warning system for infectious diseases, mandating periodical release of epidemics and preparedness for possible public health emergencies. In this regard, a nation-wide rapid information network for all notifiable infectious diseases, including SARS, has been established and maintained for real-time reports. The network covers 100% of economically developed provinces in the east, and has reached 75% of townships throughout the country. The list of monitoring has now been extended to include nearly 20 communicable diseases, compared with only a few before the SARS outbreaks. The geographical distribution of monitoring sites is also widespread. For example, the total number of sentinels for influenza and SARS throughout the country has increased from 40 before 2002, to 195 in 2007. Techniques and

strategies used are essentially consistent with World Health Organisation (WHO) recommendations and those adopted by developed countries elsewhere. China has stipulated the process and timing for reports on any emergent public health events, and made online submissions of the reports by designated centres mandatory. Via the Internet, reports on SARS and other communicable diseases, if confirmed, can be directly submitted and received within two hours.

Recently, a probable limited person-to-person transmission of highly pathogenic influenza A (H5N1) virus was reported.⁽¹⁹⁾ Thanks to the rapid information network for SARS and avian flu, the index patient with suspected H5N1 virus infection was isolated on day three of onset. Being in unprotected close exposure to the index case, his father fell ill three days after and was immediately isolated. In addition, 91 contacts with close exposure to one or both cases were quarantined. Experts from the Chinese Expert Committee against Avian Flu were requested to give consultation on the diagnosis and management. Owing to the early isolation and effective respiratory support, the father of the index case was cured, and no further evidence of human-to-human transmission was shown.

HEALTHCARE TRAINING, EDUCATION AND REINFORCEMENT

After the SARS epidemics, specialised training courses for all HCWs were provided yearly in China, with emphasis on diagnosis and reporting of infectious diseases. Over the past years, the all-round public education has made people, including government officials, more aware of communicable diseases and their impact on the economy and societal stability. The media is also actively working with healthcare institutions to promote disease prevention and management. Currently, China's government recognises the importance of, and has stepped up infrastructures in disease control, medical resuscitation and public health surveillance. For example, Guangdong Province in southern China has continuously invested more than ten billion RMB for this purpose. The funding greatly helped in improving logistics to cope with health crises, purchase of diagnostic hardwares, and more importantly, overall increased capacities to contain emerging events or other complicated situations.

MANAGEMENT

There was no compelling evidence that amantadine, oseltamivir or other antiviral agents showed efficacy in the treatment of a SARS-CoV infection. In a retrospective, matched cohort study by Chan et al, lopinavir/ritonavir (Kaletra), a protease inhibitor used in patients infected with AIDS/HIV, given as "initial treatment" in combination with ribavirin, was associated with favourable clinical outcomes.⁽²⁰⁾ The efficacy of Kaletra was further proven in the *in vitro* foetal rhesus kidney cell infected with SARS-CoV.⁽²¹⁾ Efficacy of corticosteroid remains controversial. However, in a large, retrospective study by Chen et al,

proper use of corticosteroids in confirmed critical SARS patients resulted in lowered mortality and shorter hospital stay, and was not associated with significant secondary lower respiratory infections.⁽²²⁾

With regard to influenza A (H5N1) viruses, early treatment with oseltamivir is recommended,⁽²³⁾ and data from an uncontrolled clinical trial suggests that it improves survival, although the optimal dose and duration of therapy are still uncertain. A double dose of oseltamivir (e.g. 150 mg twice daily in adults) and an increased duration of therapy for a total of ten days may be reasonable, given the high levels of N5N1 virus replication, observations of progressive disease despite early administration of standard dose oseltamivir (75 mg twice daily for five days in adults) within 1–3 days after the onset of the illness.⁽²⁴⁾ However, patients should be cautioned against the adverse effects.

There is no evidence to show that corticosteroid therapy is effective in patients with H5N1 virus infection. Corticosteroids may be beneficial for those with rapid deterioration ($IO \leq 300$) or diagnosed sepsis.⁽¹⁷⁾ Recently, with the use of convalescent plasma from a patient who had recovered from H5N1 infection four months before, we successfully treated a 31-year-old driver who developed multiorgan dysfunction six days after onset of H5N1 infection.⁽²⁵⁾ After the first transfusion, the patient's viral load dropped from 1.68×10^5 to 1.42×10^4 copies per ml and was undetectable within 32 hours, indicating that passive immunotherapy may be a viable option for the treatment of influenza A (H5N1) infection.

VACCINE DEVELOPMENT

So far, vaccination appears to be the best prophylaxis of SARS-CoV infection, even in those who are suffering from SARS. In a three-year follow-up study of IgG and neutralising antibodies, we found that the immune protection against SARS-CoV may wane over time.⁽²⁶⁾ Using RNA interference techniques, we have identified two small interfering RNA (siRNA) duplexes out of 48 siRNA candidates, which were able to relieve SARS-like symptoms, reduce SARS-CoV RNA copies and attenuate histopathological change in rhesus macaque, when administered before or during a SARS-CoV infection. Although this study is far from a clinical approach, the finding of the effective siRNA enables a massive reduction in the time needed for the development of new targeted prophylactic and therapeutic agents against SARS-CoV infection.⁽²⁷⁾

Lin et al, in a preliminary study, reported that 18 normal subjects receiving inactivated whole SARS-CoV vaccine, have shown a significant increase in neutralising antibodies 35–42 days after the vaccination, which may potentially benefit the prophylaxis of SARS-CoV infection.⁽²⁸⁾ Recently, Zhu et al identified an antibody, m396, from the fragments containing the receptor-binding domain, which is a major SARS-CoV neutralisation determinant; this might neutralise all epidemic SARS-CoV

isolates from the two SARS outbreaks and palm civets, and could have potential application for the prophylaxis and treatment of SARS-CoV infections.⁽²⁹⁾

Recently, immunogenically-inactivated, safer H5 vaccines have been developed.⁽³⁰⁾ As compared with the conventional subunit vaccine,⁽³¹⁾ the use of whole virus H5N1 vaccine was able to substantially reduce the amount of vaccine antigen required to induce immune responses in persons who have not received a priming dose, and they can induce immune responses to antigenically-drifted viruses. Thus, the possibility raised is that inactivated H5 vaccination may be capable of not only preventing persons from H5N1 virus infection, but also developing passive immunotherapy with humanised monoclonal or polyclonal antibodies.

More recently, a patient in Nanjing who developed severe H5N1 virus infection six days after unprotected bedside care of his son (index case), fully recovered by receiving two 200 ml transfusions of plasma. The plasma was donated by a 30-year-old woman who had received two doses of inactivated whole-virion H5N1 vaccine (day 0 and 28) in the phase I clinical trial group, as reported by Lin et al.⁽²⁸⁾ In addition, various approaches, including conserved antigen vaccines, vectored H5 vaccines and other adjuvants, are being developed.

REFERENCES

- Janies D, Habib F, Alexandrov B, Hill A, Pol D. Evolution of genomes, host shifts and the geographic spread of SARS-CoV and related coronaviruses. *Cladistics* 2008; 24:111-30.
- Wong S, Lau S, Woo P, Yuen KY. Bats as a continuing source of emerging infections in humans. *Rev Med Virol* 2007; 17:67-91.
- Ng PC, Li K, Wong RP, et al. Proinflammatory and anti-inflammatory cytokine responses in preterm infants with systemic infections. *Arch Dis Child Fetal Neonatal Ed* 2003; 88: F209-13.
- Julkunen I, Melén K, Nyqvist M, et al. Inflammatory responses in influenza A virus infection. *Vaccine* 2000; 19: S32-S37.
- Luster AD. Chemokines--chemotactic cytokines that mediate inflammation. *N Engl J Med* 1998; 338:436-45.
- Jiang Y, Xu J, Zhou C, et al. Characterization of cytokine/chemokine profiles of severe acute respiratory syndrome. *Am J Respir Crit Care Med* 2005; 171:850-7.
- Xu J, Zhong S, Liu J, et al. Detection of severe acute respiratory syndrome coronavirus in the brain: potential role of the chemokine mig in pathogenesis. *Clin Infect Dis* 2005; 41:1089-96.
- Agostini C, Facco M, Siviero M, et al. CXC chemokines IP-10 and mig expression and direct migration of pulmonary CD8+/CXCR3+ T cells in the lungs of patients with HIV infection and T-cell alveolitis. *Am J Respir Crit Care Med* 2000; 162:1466-73.
- von Andrian UH, Mackay CR. T-cell function and migration. Two sides of the same coin. *N Engl J Med* 2000; 343:1020-34.
- Cheung CY, Poon LL, Lau AS, et al. Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: a mechanism for the unusual severity of human disease? *Lancet* 2002; 360:1831-7.
- Lipatov AS, Andreansky S, Webby RJ, et al. Pathogenesis of Hong Kong H5N1 influenza virus NS gene reassortants in mice: the role of cytokines and B- and T-cell responses. *J Gen Virol* 2005; 86:1121-30.
- de Jong MD, Simmons CP, Thanh TT, et al. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nat Med* 2006; 12:1203-7.
- World Health Organization. WHO guidelines for the global surveillance of severe acute respiratory syndrome (SARS). Updated recommendations, October 2004. Available at: www.who.int/csr/resources/publications/WHO_CDS_CSR_ARO_2004_1/en/index.html. Accessed May 3, 2008.
- Ho HT, Chang MS, Wei TY, et al. Colonization of severe acute respiratory syndrome-associated coronavirus among health-care workers screened by nasopharyngeal swab. *Chest* 2006; 129:95-101.
- Che XY, Qiu LW, Pan YX, et al. Sensitive and specific monoclonal antibody-based capture enzyme immunoassay for detection of nucleocapsid antigen in sera from patients with severe acute respiratory syndrome. *J Clin Microbiol* 2004; 42:2629-35.
- World Health Organization. Collecting, preserving and shipping specimens for the diagnosis of avian influenza A (H5N1) virus infection. Guide for field operations. Available at: www.who.int/csr/resources/publications/surveillance/WHO_CDS_EPR_ARO_2006_1/en/. Accessed December 20, 2007.
- Consultation Committee of China Ministry of Public Health, China Center for Disease Control, Chinese Medical Association. China guidelines of the diagnosis and management of highly pathogenic avian influenza A (H5N1). *Natl Med J* 2008; 88 (in press).
- McNeil Jr DG. Immediate treatment needed for bird flu cases, study says. *The New York Times*, September 11, 2006. Available at: www.nytimes.com/2006/09/11/world/11flu.html. Accessed May 3, 2008.
- Wang H, Feng Z, Shu Y, et al. Probable limited person-to-person transmission of highly pathogenic avian influenza A (H5N1) virus in China. *Lancet* 2008; 371:1427-34.
- Chan KS, Lai ST, Chu CM, et al. Treatment of severe acute respiratory syndrome with lopinavir/ritonavir: a multicentre retrospective matched cohort study. *Hong Kong Med J* 2003; 9:399-406.
- Chu CM, Cheng VC, Hung IF, et al. Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. *Thorax* 2004; 59:252-6.
- Chen RC, Tang XP, Tan SY, et al. Treatment of severe acute respiratory syndrome with glucocorticoids: the Guangzhou experience. *Chest* 2006; 129:1441-52.
- World Health Organization. WHO rapid advice guidelines on pharmacological management of humans infected with avian influenza A (H5N1) virus. 2006. Available at: www.who.int/medicines/publications/WHO_PSM_PAR_2006.6.pdf. Accessed December 20, 2007.
- World Health Organization. Clinical management of human infection with avian influenza A (H5N1) virus. Updated advice 15 August 2007. Available at: www.who.int/csr/disease/avian_influenza/guidelines/ClinicalManagement07.pdf. Accessed May 3, 2008.
- Zhou B, Zhong N, Guan Y. Treatment with convalescent plasma for influenza A (H5N1) infection. *N Engl J Med* 2007; 357:1450-1.
- Cao WC, Liu W, Zhang PH, Zhang F, Richardus JH. Disappearance of antibodies to SARS-associated coronavirus after recovery. *N Engl J Med* 2007; 357:1162-3.
- Li BJ, Tang Q, Cheng D, et al. Using siRNA in prophylactic and therapeutic regimens against SARS coronavirus in Rhesus macaque. *Nat Med* 2005; 11:944-51.
- Lin JT, Zhang JS, Dong XP, et al. Safety and immunogenicity of an inactivated adjuvanted whole-virion influenza A (H5N1) vaccine: a phase I randomised controlled trial. *Lancet* 2006; 368:991-7.
- Zhu Z, Chakraborti S, He Y, et al. Potent cross-reactive neutralization of SARS coronavirus isolates by human monoclonal antibodies. *Proc Natl Acad Sci U S A* 2007; 104:12123-8.
- World Health Organization. Third WHO meeting on evaluation of pandemic influenza prototype vaccines in clinical trials, 15-16 February 2007, WHO, Geneva. Available at: www.who.int/vaccine_research/diseases/influenza/meeting_150207/en/. Accessed May 3, 2008.
- Olsen SJ, Ungchusak K, Sovann L, et al. Family clustering of avian influenza A (H5N1). *Emerg Infect Dis* 2005; 11:1799-801.

The 2008 SMA Lecture was delivered on May 10, 2008 at the Suntec Convention Centre. The citation of Professor Zhong Nanshan was delivered by Dr Wong Chiang Yin, President of the Singapore Medical Association. A copy of the citation was published in the July 2008 issue of the SMA News.