EDITORIAL

Avian influenza A/H5N1 virus: management in human and bird

The high mortality of over 50% in Hong Kong patients with pneumonia caused by the influenza A/H5N1 virus in 1997 was found to be quite consistent in subsequent outbreaks in South-East Asian countries.[1,2] Our initial clinical observation was that this disease was not simply a viral pneumonia, and that all other major organs could be affected due to a cytokine storm caused by virus-induced aberrant immune activation. Fatalities were often associated with severe lymphopaenia, pancytopaenia, impaired coagulation profiles, impaired liver and renal functions in addition to oxygen desaturation on admission. Besides diffuse alveolar damage in the lungs, lymphoid atrophy and necrosis were prominent in the spleen and lymph nodes with reactive haemophagocytosis, also evident in bone marrow.[3] Unlike seasonal influenza caused by the human virus, which usually can only be isolated in the respiratory secretions, the A/H5N1 virus can also be found in the blood, faeces, and cerebrospinal fluid.[4] Thus, this so-called cytokine storm could be the end result of uncontrolled systemic viral infection as in severe septic shock due to poorly treated Gram-negative bacteraemia.

Human vaccination to prevent the A/H5N1 virus is not commercially viable because of the low number of human cases and the rather rapid viral antigenic drift. The option of antiviral therapy is very limited, because resistance to adamantanes is widespread in A/H5N1 isolates from Vietnam and Thailand. Zanamivir is only likely to be useful for prophylaxis in health care workers, because it is delivered by inhalation and not expected to reach therapeutic concentrations in extrapulmonary tissues or hypoventilated areas of lung consolidation. Treatment with oseltamivir did not obviously result in improved survival, but there was a trend towards better survival if given early in the course of illness.[5] Though the poor response may have resulted from delayed treatment initiation, other factors might be equally important. These include: the non-specific initial manifestations of A/H5N1 infection, the high initial viral load, poor oral bioavailability of oseltamivir in seriously ill patients, lack of a parenteral preparation, and the ready emergence of resistance. Since the lymphopaenia and serum pro-inflammatory cytokine levels correlate directly with the viral load in respiratory secretions,[6] it is also reasonable to consider giving immunomodulators to dampen the cytokine storm. However, the use of steroids did not improve survival and was associated with significant complications such as hyperglycaemia and superinfection.[7] In fact, after knockout of pro-inflammatory chemokine and cytokine genes or treatment with steroids, A/H5N1 virus–infected mouse models showed no significant improved survival.[8] Due to the low incidence of this important disease, randomised controlled clinical trials are unlikely to be conducted. However, data from mice models suggest that high dose of oseltamivir therapy prolonged to more than 8 days,[9] combination of oseltamivir with amantadine,[10] and use of high titres of neutralising monoclonal antibody or convalescent plasma, may improve survival.[11] Recently, we combined the systemic administration of zanamivir with the COX-2 inhibitor celecoxib and mesalazine to treat mice inoculated with a high dose of A/H5N1 virus.[12] Despite delayed therapy initiation of up to 48 hours after inoculation, this combination significantly reduced the viral load, production of pro-inflammatory cytokines, chemokines, leukotrienes, as well as mortality. The inhibitory activities of these non-steroidal anti-inflammatory agents against the pro-inflammatory response, together with the anti-apoptotic activities of the aminosalicylate, reduced cell death and tissue damage in the host. The concomitant use of an effective antiviral is essential, not only to limit the extent of viral replication that drives the cytokine dysfunction triggered by the infection, but also to counteract the possible increase in viral load after COX-2 inhibition. Notably, these drugs are widely available and intravenous zanamivir has been used in humans with little in the way of side-effects.[13-15]

However, prevention is always better than cure. No developed nation in the world is really prepared for a 1918-like pandemic influenza. In the absence of efficient inter-personal spread of the A/H5N1 virus, preventing major outbreaks of human infection relies on controlling its endemicity in poultry. This entails prevention and prompt management of outbreaks in poultry, separation of poultry from humans to minimise transmission to them, and proper management of occasional human infections. At the height of the 1997 outbreak in Hong Kong, 20% of the poultry in wet markets were infected by the virus. Control of the outbreak ensued after culling of all the 1.5 million poultry throughout Hong Kong. Sale of live ducks and geese in wet markets was banned, as these birds can shed the virus asymptomatically. Biosecurity measures in local farms were strictly enforced, and a bi-weekly rest day with cleansing of all the poultry stalls was introduced to interrupt the transmission cycle in wet markets. Vaccination against influenza A/H5 infection was required for all poultry in local farms and farms supplying live poultry to Hong Kong from Mainland

China. These stringent measures appeared successful in preventing the incursion of the virus into local farms and markets for several years. Unfortunately, we cannot prevent the expected antigenic drift which will overcome the protection conferred by the poultry vaccine and thus require changes in vaccine according to the dominant endemic viral strain at that time. Complete elimination of illegal poultry imports into Hong Kong from unregistered farms in the Mainland is unlikely to be successful. Moreover, chicken stalls in wet markets may be regarded as mini-farms, where biosecurity measures comparable to those imposed on recognised farms are impossible to implement. Thus, the final answer depends on central slaughtering, which eliminates any potential contact of live poultry with the general population. In the interim before central slaughtering is launched, daily culling of all unsold chickens can be expected to stop viral shedding from newly infected chickens. The latter may not stay in the market long enough to exceed the incubation period for viral shedding. However, such measures cannot stop viral shedding from illegally imported infected chickens.

Control of avian influenza outbreaks in poultry in developing countries poses even more formidable problems. The rising demand for meat protein associated with the improving open-door economy in South-East Asia is responsible for a tremendous increase in poultry farming. Regrettably, no corresponding improvement in the biosecurity measures have followed in the ensuing profligation of farms and markets, and over half of such poultry are reared in backyard premises. Theoretically, country-wide veterinary and virological surveillance of birds, perimetric depopulation of infected zones, and targeted immunisation of poultry with correct vaccines could all be helpful. Other potentially useful measures include: segregation of poultry species, regular moratoria of poultry in the markets, and the implementation of biosecurity and hygienic practices in farms, markets, and at a personal level might also help to control poultry pandemic. How many of these measures are practicable is questionable. Alternatively, lesser scale interventions at the district level can be considered in response to local virus detection even without evidence of excess poultry deaths, since virus shedding is common in asymptomatic water fowl. To reduce the environmental viral load and therefore the risk of re-infection of farmed poultry, a planned one-off moratorium of 3 weeks during the hottest months of the year may be an important measure, as shown by mathematical modelling.[16] Backyard farms will then be re-populated by hatchlings from virus-free chickens and minor poultry to ensure a virus-free environment. Universal immunisation against avian influenza of all poultry in backyard farms is not feasible, and hence immunisation should be preferentially targeted to ducks, geese, and chickens in industrial farms. Free grazing of ducks and geese outside the pens should only be allowed if the birds carry adequate titres of neutralising antibodies against H5.

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A/H5N1禽流感病毒：人和禽的處理

1997年香港因A/H5N1流感病毒致患肺炎的病人，死亡率超過50%，而東南亞國家後來的情況與此相當一致。據我們的初期觀察，這種疾病並不僅僅是一種病毒性肺炎，由病毒引起的免疫系統異常活躍而導致的細胞激素風暴，也可能影響到所有其他重要器官。致命的原因，往往與入院時淋巴細胞嚴重減少、全血細胞減少、凝血狀態損毀、肝和腎功能損毀，以及氧飽和度下降有關。除了廣泛的肺泡受損之外，淋巴萎縮和壞死的情況在帶有反作用的血球被吞噬現象的胰臟和淋巴結之中，乃至在骨髓裏，相當顯著。與通常只能存在於呼吸道分泌物內的人類病毒所導致的季節性流感不同，A/H5N1病毒可以在血液、糞便和腦脊液中找到。因此，這種所謂的細胞激素風暴只不過是一種失控的系統性病毒感染的預期結果，情況正如革蘭氏陰性菌血症處理不善時引致的敗血症一樣。

由於人類的感染個案不多，以及病毒抗原的迅速漂變，所以接種防止A/H5N1病毒的疫苗並不符合商業原則。抗病毒治療的方法不多，因為越南和泰國分離出來的A/H5N1對於金剛胺有廣泛的抗藥性。扎那米韋只有利於醫護人員作預防，因為這種藥物是經口腔吸入，而且不能夠達到被肺炎過程實變了或換氣不足的肺部或肺外組織。而奧斯他韋治療沒有明顯提高病者存活的機會，但倘若在發病初期便使用這種藥物，則存活的機會較大。對奧斯他韋反應不佳可能是延緩開始用藥所致，但其他的因素可能同樣重要，這些因素包括A/H5N1感染初期病徵不明確、初期病毒量高、嚴重病者口服奧斯他韋的吸收率低及沒有靜脈注射的方劑、以及治療期間產生的抗藥性。由於淋巴細胞減少和血清促炎細胞因子的水平，與呼吸道分泌物的病毒量直接相關，所以除了給予抗病毒藥物之外，加入免疫調節劑以減低細胞激素風暴，也是合理的處理辦法。然而，使用類固醇沒有提高病者的存活機會，反而會引起血糖過高和重複感染等併發症。事實上，受病毒感染被剔除了促炎因子和細胞因子基因的實驗鼠，或接受類固醇處方後，存活機會也沒有明顯提高。由於這種重要疾病的發病率低，不大可能進行隨機的監控臨床試驗。不過，從實驗鼠得到的數據顯示，較高劑量的奧斯他韋和延長至8天以上的治療期、奧斯他韋與金剛胺的結合使用，以及使用高滴度中和單克隆抗體或康復血漿，應可提高病者的存活機會。近期，我們把扎那米韋用腹腔注射作治療受高劑量A/H5N1病毒的實驗鼠，再配合COX-2抑制劑塞來昔布和美沙拉嗪。儘管治療延緩至病毒注射後48小時才開始，這種結合療法顯著減少了病毒量，促炎細胞因子、趨化因子和白三烯的產生，以及動物的死亡。這些非類固醇抗炎劑對抗促炎反應的抑制力，加上氨基水楊酸鹽的抗凋亡力，減少了宿主的細胞死亡和組織損毀。由自然感染形成的病毒複製，引致細胞功能障礙，因此必須伴隨使用有效的抗病毒藥物，以限制病毒的複製程度。除此以外，這些藥物也用以抗衡COX-2抑制作用後病毒量的上升。還有應該指出的是，這些藥物供應充足，而且扎那米韋靜脈注射在人體內副作用不多。

預防永遠勝於治療，但世界上沒有一個已發展國家真正為1918年那樣的流感做好準備。在A/H5N1病毒還沒有出現有效人傳人之前，預防人類感染大爆發的措施，主要是監控病毒發現地點、預防和迅速處理家禽的爆發、人禽分隔以減少禽傳人的可能，以及適當處理人類的偶發感染。1997年香港禽流感高峰期時，濕貨市場20%家禽受到感染。控制措施是宰殺全香港全部150萬隻家禽；濕貨市場禁售活鴨活鵝，因為這些家禽可以在毫無症狀的情況下排放病毒；本地農場嚴格執行生物安全措施；濕貨市場家禽攤檔每兩周實施一天清潔消毒；供應活家禽的所有本地和大陸農場均須進行A/H5流感防疫注射。這些嚴格的措施多年來有效防止了病毒入侵本地農場和市場。可惜，預期的抗原漂變無法阻止，而可能導致家禽疫苗的保護力下降。另外，非法入口的家禽之中可能有來自大陸非註冊農場的受感染家禽，但要完全消滅這些非法入口亦困難重重。重要的是，濕貨市場的活雞攤檔實際上是個小農場，由於空間所限，不可能實施農場的生物安全措施。因此，最後的答案便落在中央屠宰之上，唯有這樣才可避免活家禽與公眾接觸。在中央屠宰實行之前的過渡時期，活雞不過夜的做法可以阻止新感染雞隻的病毒排放，避免病毒在逗留街市期間經歷充足的潛伏期。但是，這種做法不能防止已受感染的非法入口雞隻的病毒排放。

發展中國家控制家禽爆發禽流感，面對更為嚴峻的問題。東南亞的經濟不斷改善，帶來對禽肉蛋白質需求的不斷上升，為了應付這個需求，只有大量增加家禽飼養。可是，農場和市場的生物安全措施卻沒有相應改善，而半數家禽是在家居後院的農場上飼養的。理論上，全國範圍的禽鳥和病毒監控，在疫區周邊宰殺可能受感染的家禽，使用適當的疫苗作防疫，分隔禽類，市場定期禁止售賣家禽，在農場、市場和個人層面實行生物安全和衞生措施，凡此種種皆足以控制這種禽鳥傳染病。但在發展中國家這些措施卻很多時候未必可行。如果在病毒檢測中沒有過量的家禽死亡，則在地區層面上可以考慮採取規模較小的措施，因為病毒排放在無症狀的水禽中相當常見。為了降低環境的病毒量，從而減少農場家禽再次感染的風險，那麼按照數學模型的計算結果，在年中最炎熱的月份一次過禁售三周，也許是一種重要的措施。後院農場事後將重新飼養來自無病毒雞隻和次要家禽的幼苗，以保證環境不受病毒污染。給後院農場所有家禽做全面的禽流感免疫工作並不可行，因此，免疫工作應先針對工業農場的鴨、鵝、雞。而只有在這些禽鳥帶有足夠滴度的抗H5病毒中和抗體的時候，才可允許鴨鵝在欄外自由走動。