Pathology and Pathogenesis of 2009 Pandemic H1N1 Influenza

Wun-Ju Shieh, MD, MPH, PhD
Infectious Diseases Pathology Branch, Division of Viral and Rickettsial Diseases
Centers for Disease Control and Prevention

BACKGROUND

Novel influenza A (H1N1) is a new influenza virus of swine origin that first caused illness in Mexico and the United States in March and April, 2009. The novel influenza A (H1N1) virus spreads in the same way as regular seasonal influenza viruses do, mainly through the coughs and sneezes of people who are sick with the virus, but it may also be spread by touching infected objects and subsequently touching one’s nose or mouth. Novel H1N1 infection has been reported to cause a wide range of flu-like symptoms, including fever, cough, sore throat, body aches, headache, chills and fatigue. In addition, many people also have reported nausea, vomiting and/or diarrhea. The first novel H1N1 patient in the United States was confirmed by laboratory testing at CDC on April 15, 2009. The second patient was confirmed on April 17, 2009. It was quickly determined that the virus was spreading from person-to-person. On April 22, CDC activated its Emergency Operations Center to better coordinate the public health response. On April 26, 2009, the United States Government declared a public health emergency and has been actively implementing the nation’s pandemic response plan.

On June 11, 2009, the World Health Organization (WHO) indicated that a global pandemic of novel influenza A (H1N1) was underway by raising the worldwide pandemic alert level to Phase 6. This action was a reflection of the magnitude of spread of the new H1N1 virus, and not the severity of illness caused by the virus. At that time, more than 70 countries had reported cases of novel influenza A (H1N1) infection. By June 19, 2009, all 50 states in the United States, the District of Columbia, Puerto Rico, and the U.S. Virgin Islands had reported novel H1N1 infection.

Since the WHO declaration of a pandemic, the new H1N1 virus has continued to spread, with the number of countries reporting cases of novel H1N1 nearly doubling. In the Southern Hemisphere’s, regular influenza season started concurrently with the new H1N1 influenza in June. In the United States, significant novel H1N1 illness has continued to occur in the summer,
with localized and in some cases intense outbreaks. The United States continues to report the largest number of novel H1N1 cases of any country worldwide, however, most people who contracted novel H1N1 have recovered completed without requiring medical treatment.

Given ongoing novel H1N1 activity to date, the CDC anticipates that there will be more cases, more hospitalizations and more deaths associated with this pandemic in the United States over the winter and early spring. The novel H1N1 virus, in conjunction with regular seasonal influenza viruses, poses a potential threat to the general public due to the significant illness it may cause, especially with associated hospitalizations and deaths during the influenza season. The mortality rate of novel H1N1 is estimate to be around 0.01%. This presentation describes the clinicopathologic, epidemiological, and pathogenetic studies of autopsy tissue specimens obtained from U.S. fatal cases with 2009 pandemic influenza A (H1N1) virus infection.

**PATIENT CHARACTERISTICS AND LABORATORY TESTS**

One hundred confirmed case-patients with fatal 2009 H1N1 virus infection were evaluated at Infectious Disease Pathology Branch, CDC from May 12 to October 1, 2009. Fifty-three (53%) of these case-patients were confirmed to have 2009 H1N1 by testing postmortem specimens. These 53 cases had no antemortem diagnosis and the confirmatory diagnosis was only obtained from testing postmortem samples. This finding underscores the important role of medical examiners, coroners, and pathologists in infectious disease surveillance by performing postmortem examination and testing autopsy samples.

The median age of fatal case-patients was 36 years, range 2 months to 84 years, 80% were aged 20 - 60 years, and 51 (51%) were male. The majority (85%) of case-patients with known previous medical history had at least one underlying comorbidity. Obesity (46%), cardiovascular disease (25%) and asthma (22%) were the three most frequent conditions reported. Fever (82%), cough (67%) and shortness of breath (58%) were the most common signs and symptoms reported, with a median duration from illness onset to death of 8 days, range 1 to 44 days. Fifty-eight (67%) of 87 case-patients with available clinical history were hospitalized prior to death. Forty-two (74%) of 57 case-patients with available hospital records required mechanical ventilation. Radiographic diagnosis of pneumonia was documented in 59% (38/64) of case-patients.
In the cases with known medical history, obesity (BMI ≥ 30) is the most significant associated factor and almost 19% of these patients were extremely obese (BMI ≥ 40). There is a clear association between obesity and metabolic disorders; however, very little is known about the effect of obesity on immune function, especially during an infection. It was reported that diet-induced obese mice are more susceptible to morbidity and mortality during influenza infection than lean mice. Obesity may interfere with cellular responses during influenza infection, leading to selective impairment in dendritic cell function and alterations in the T-cell population that may be detrimental to the host. Whether similar altered immune responses also occurred in humans with influenza virus infection are unknown. Nevertheless, obesity is the most common precipitating factor for obstructive sleep apnea as well as a risk factor for the development of asthma. A number of studies also indicate its association with a higher risk of developing deep vein thrombi, pulmonary emboli, pulmonary hypertension, and pneumonia. Further studies are needed to elucidate the pathophysiologic affect of obesity and other medical conditions on patients with 2009 pandemic influenza A (H1N1) virus infections.

The autopsy samples were evaluated with histopathologic examination, immunohistochemical assays (IHC), PCR assays, viral culture, and electron microscopic examination. Of the 100 case-patients with confirmed 2009 H1N1, testing of respiratory tissue by rRT-PCR assays at CDC were positive for influenza A virus in 90, including 87 for H1N1 and 3 non-subtypeable virus. Based on available records for 80 case-patients with known duration of illness, rRT-PCR results were positive for 2009 H1N1 in respiratory tissue specimens of 42 (53%) case-patients with illness duration <10 days and in 28 case-patients (35%) with illness >10 days when death occurred. Negative rRT-PCR results for 2009 H1N1 were not observed in any case-patients with known illness duration <10 days, but results were negative in 7 case-patients (9%) with duration >10 days. In these same 80 case-patients with known duration of illness, positive IHC results for influenza A viral antigen were observed in respiratory tissues of 31 case-patients (39%) with illness duration <10 days and in 5 case-patients (6%) with illness >10 days. In contrast, negative IHC results for influenza A were observed in respiratory tissues of 14 case-patients (18%) with time from onset to death <10 days and in 31 case-patients (39%) who died after an illness >10 days. 2009 H1N1 virus was isolated from fresh lung tissue specimens in five of 30 case-patients tested.
HISTOPATHOLOGIC FINDINGS

The major histopathologic features were observed in airways and lungs. Eighty-five case-patients had airway tissues available for evaluation. The most frequent histopathologic findings in airways were inflammation and edema (66%). The inflammation was usually mild and consisted predominantly of mononuclear cells. Necrosis of epithelium (26%) and hemorrhage (18%) were less frequently observed. Lung tissues in all case-patients showed a spectrum of histopathologic changes of diffuse alveolar damage (DAD). The nature and extent of DAD generally corresponded to the duration of clinical illness of the patients. Forty-one case-patients had paratracheal or hilar lymph nodes available for evaluation and hemophagocytosis was noted in 25 (61%) of these case-patients. Pulmonary thromboemboli were noted in gross autopsy findings or microscopically in 17 case-patients. No histopathologic evidence of myocarditis or encephalitis was observed in any of the case-patients with heart (n=30) or CNS samples (n=19) available for evaluation. Histopathologic findings in other organs were nonspecific and most likely associated with the patients’ underlying medical conditions. These findings included prominent eosinophils in patients with history of asthma; enlarged nuclei of cardiac myocytes in patients with history of hypertension; and fatty metamorphosis in the liver in obese patients.

Histopathologic evaluations accentuated DAD as the most significant and consistent finding. This lung involvement is more similar to the histopathologic features in fatal avian influenza (H5N1) and is seen less frequently in fatal seasonal influenza cases. However, a proportion of pandemic influenza A (H1N1) cases in this report also showed inflammation or other histopathologic changes in trachea, bronchi, or bronchioles, a pattern more commonly observed in severe or fatal cases of seasonal influenza.

Other respiratory viral infections can also cause DAD, especially at the end stage of critical illness. Some of these viruses may show distinct cytopathic effects or inclusions in the lung that can be differentiated from influenza virus infection. Examples of these include adenovirus, human herpesviruses, measles virus, respiratory syncytial virus, Nipah virus, and parainfluenza virus. Since these distinct cytopathic effects are not seen in influenza virus infection, a combination of clinical judgment, epidemiological surveillance data, and various laboratory tests are necessary for a reliable diagnosis of influenza.

VIRAL LOCALIZATION AND CELLULAR TARGETS
By using IHC assay with anti-influenza A NP antibody, the viral antigens were observed in respiratory tissues from 44 case-patients (44%); the amount of influenza virus antigen varied, with abundant immunostaining in 9 and rare in 24 case-patients. Viral nucleoprotein antigens were localized in the nuclei and cytoplasm of infected cells, including epithelial cells in airways, submucosal glands, and pneumocytes, either detached or lining alveoli. Antigens were also seen in association with hyaline membranes and in endothelial cells in rare cases. Double staining revealed that the major cellular targets of viral infection were pneumocytes, predominantly type II, and occasionally macrophages. No immunostaining of influenza A viral antigen was detected in any of the non-respiratory tissue samples available. Influenza rRT-PCR testing on a limited number of these non-respiratory samples was also negative. Electron microscopic examination of lung tissue identified rare infected cells with extracellular virus particles in the alveolar space. Virions were round to oblong-shaped and averaged 88 nm in diameter; some particles were surrounded by spikes approximately 12 nm in length.

Immunolocalization showed viral antigens were predominantly in the lung parenchyma. The damage to the lung is associated with localization of viral antigens. The amount of viral antigens varied from case to case, and appeared to be more abundant in cases with shorter duration of illness. This temporal correlation probably denotes the clearance of viral antigens by host immune responses at later stage of illness. IHC with double immunostaining for influenza virus and cell markers, such as surfactant, cytokeratin, and CD68, demonstrated the localization of pandemic influenza A (H1N1) virus antigens is mainly in pneumocytes, especially type II. The involvement of pneumocytes is similar to avian influenza (H5N1) virus infections and other viral infections like SARS, representing the hallmark of severe damage of the alveolar architecture. Type II pneumocytes are known to secrete pulmonary surfactant, which reduces surface tension and preserves the integrity of the alveolar space. These cells also play an important role in tissue restitution after lung damage. Type II pneumocytes can be directly infected by bacteria or viruses and modulate the corresponding pathogenesis of these organisms. Comparing to avian influenza (H5N1) virus infection, the involvement of pneumocytes with pandemic influenza A (H1N1) virus was much more extensive. This observation correlates with the studies of receptor tropism among different influenza A viruses and probably represents more accessible receptors in human lung for pandemic influenza A (H1N1) virus. Viral antigens can also be seen in tracheo-bronchiolar epithelial cells, glandular epithelial cells, and occasional
macrophages. The viral immunolocalizations in airway and lung parenchyma are in agreement with studies of receptor binding demonstrating the ability for 2009 H1N1 virus to target both upper and lower respiratory tract tissue. In cases of seasonal influenza, viral antigens are predominantly present in airway epithelial cells and rarely involve alveolar pneumocytes or macrophages. The differential presence of receptors may account for the dissimilarity of histopathologic changes and viral antigen distribution among seasonal influenza A viruses, avian influenza A (H5N1) virus, and 2009 pandemic influenza A (H1N1) infections.

**BACTERIAL AND VIRAL COINFECTIONS**

Overall, 26% of case-patients had confirmatory test results of bacterial coinfection. Twenty-nine case-patients showed histopathologic evidence of bronchopneumonia with prominent alveolar polymorphonuclear cells, indicating a possibility of bacterial coinfection. Of these, 22 (76%) case-patients had a specific bacterial pathogen identified. Bacterial agents were identified in an additional 4 case-patients that did not show histopathologic evidence of bronchopneumonia in the tissue sections examined. Gram-positive cocci were the most frequent bacteria identified by using special stains. IHC and PCR assays were positive as follows: 9 case-patients positive for S. pneumoniae, 3 case-patients for S. pyogenes, 1 case-patient for both S. pyogenes and S. pneumoniae, 1 case-patient for both S. pyogenes and S. mitis, 1 case-patient for S. mitis, 1 case-patient for S. agalactiae, 4 case-patients for MRSA, 1 case-patient for both MRSA and S. pyogenes, 1 case-patient for both MRSA and H. influenzae, and 4 case-patients for MSSA. None of the case-patients were found to have evidence of a coinfection with RSV, parainfluenza viruses 1-3, or adenovirus.

Bacterial co-infections in severe cases of influenza have been well documented in previous influenza pandemics and in studies of seasonal influenza. For instance, during the 1957-1958 H2N2 pandemic, secondary bacterial pneumonia was considered the most frequent complication of influenza with an occurrence in <75% of fatal cases. Interactions among influenza virus and co-infecting bacterial pathogens often affect the nature and severity of clinical manifestations and disease outcome. As previously reported, it is often difficult to correlate culture results with clinicopathologic features of pneumonia due to multiple confounding situations, such as inconsistency of obtaining samples for culture, contamination of postmortem samples with growth of mixed bacteria, and inherent problems of culture techniques.
IHC assays performed using formalin-fixed, paraffin-embedded tissues offered the advantage of identifying specific respiratory bacteria pathogens in areas with histopathologic evidence of bacterial pneumonia. The IHC positive cases can be furthered confirmed by paneubacterial 16s PCR assay and agent-specific PCR assay. In this series, bacterial pneumonia was present in 29% of cases, with S. pneumoniae, S. aureus, and S. pyogenes as the most frequent agents identified, alone or combined with other organisms. The incidence of S. aureus, either MRSA or MSSA, has become alarmingly high, especially among younger patients.

**PATHOGENESIS**

The consistent presence of DAD in all of the fatal pandemic H1N1 Influenza cases indicates a primary cytopathic effect caused by viral infection. Although immunolocalization does not indicate active viral replication, the finding of viral antigen within pneumocytes and in association with hyaline membranes suggests a direct viral cytopathic effect as a major pathogenic mechanism in this disease. In addition to the primary viral pneumonia, other factors, such as underlying medical conditions and bacteria co-infections as discussed previously may also contribute to the morbidity and mortality of this novel virus infection. Various degrees of hemophagocytosis is present in 15 (65%) of 23 cases with lymph nodes available for examination. Hemophagocytosis is a nonspecific histopathologic finding that can be seen in many infectious diseases. However, it may play a role in pathogenesis of influenza virus infection because of its possible correlation with cytokine-mediated process. Several studies of naturally acquired or experimentally infected influenza demonstrated that respiratory and constitutional symptoms correlate with the presence of multiple cytokines, including IL-4, IL-6 and TNF-α, in plasma or nasopharyngeal fluid, but viremia has not been detected through the disease process. The potential role of these cytokines in the pathogenesis of developing severe or fatal outcome of influenza virus infection needs further investigation.

In summary, the most significant histopathologic feature in fatal cases of 2009 pandemic influenza A (H1N1) is various degrees of diffuse alveolar damage. PCR and IHC assays are instrumental in establishing diagnosis and studying pathogenesis of this emerging viral infection. The immunolocalization of pandemic influenza A/H1N1 viral antigens shows pneumocytes and alveolar lining cells are the most prominent targets involved in the infection. Although there are
limitations for detecting bacteria in postmortem lung samples, a combination of histopathologic evaluation, special stains, IHC assays, and PCR assays provides valuable diagnostic information to identify etiologic bacterial organisms as the source of co-infection. In addition to bacterial co-infection, the severe or fatal outcome of many patients with pandemic influenza A (H1N1) virus infection may be attributed to other underlying medical conditions, such as obesity and asthma. More studies, including experimental animal studies, are needed to further our understanding in the pathogenesis of this emerging influenza virus.

REFERENCES


PATHOGENESIS OF RICKETTSIAL DISEASES

By David H. Walker

Human rickettsioses are caused by numerous named pathogens: *Rickettsia rickettsii* (Rocky Mountain spotted fever), *R. conorii* (Mediterranean spotted fever), *R. africae* (African tick bite fever), *R. akari* (rickettsialpox), *R. sibirica* (North Asian tick typhus and lymphangitis-associated rickettsiosis), *R. australis* (Queensland tick typhus), *R. japonica* (Japanese spotted fever), *R. honei* (Flinders Island spotted fever), *R. prowazekii* (epidemic typhus), *R. typhi* (murine typhus), and several emerging unnamed diseases (*R. massiliae, R. aeschlimannii, R. monacensis, R. helvetica,* and *R. amblyommii*). Rickettsiae are small obligately intracellular gram negative bacteria that reside in an arthropod host as its ecologic niche during at least a portion of their natural history. They have small genomes resulting from reductive evolution and rely upon the host cell for synthesis of numerous nutrients and building blocks for growth.

Rickettsiae are transmitted in the saliva of infected ticks and mites or feces of infected fleas and lice, and spread via lymphatic vessels to the regional lymph nodes and hematogenously to endothelium throughout the body. Life-threatening lesions are interstitial pneumonia/noncardiogenic pulmonary edema and meningoencephalitis associated with extensive rickettsial infection of pulmonary and cerebral endothelium. The pathologist may observe only subtle perivascular edema prior to the onset of adaptive immunity. The perivascular infiltration of mononuclear cells actually comprises the CD8 and CD4 T lymphocytes and macrophages that mediate immune clearance of the pathogen.

The crucial pathophysiologic effect of rickettsial endothelial infection is increased microvascular permeability resulting from discontinuities in interendothelial adherens junctions, the effects of TNFα, IFNy, IL-1β, and VEGF, and COX-2 dependent production of PGE2 and PGI2. The pathogenic mechanisms of endothelial injury include endothelial cell production of toxic reactive oxygen species, damage to the cell membrane upon rickettsial exit, and cytotoxic T lymphocyte-induced apoptosis of infected endothelial cells. Rickettsial infections cause a procoagulant state, but only very rarely disseminated intravascular coagulation. Thrombi comprise non-occlusive hemostatic plugs that are appropriately located at foci of severe endothelial damage and prevent severe hemorrhage and rarely cause ischemic necrosis. Host factors play an important role in severity of illness, including older age, male gender, G6PD deficiency, diabetes, alcoholism, and IFNy SNP genetic polymorphism.
Rickettsiae adhere to target cells by outer membrane autotransporter proteins OmpA, OmpB, Sca1, and Sca2. Ku70 is a host cell receptor for OmpB, triggering actin rearrangement and induced phagocytosis. Rickettsiae rapidly escape from the phagosome, most likely via secretion of membranolytic phospholipase D and hemolysin C. Spotted fever group rickettsiae effect host actin-based mobility through rickettsial proteins RickA and Sca2 with actin tails propelling them into filopodia for extracellular release or cell-to-cell spread. Rickettsial manipulation of its host cell also includes activation of NF-kB, which inhibits apoptosis, prolonging the life of the rickettsial niche.

Innate immunity is triggered by TLR4-dependent activation of dendritic cells that, in turn, activate NK cells to produce infection-dampening IFNγ. Adaptive immunity is effected by cytokine-activated endothelial cells, macrophages, and hepatocytes which kill intracellular rickettsiae in association with autophagy by producing nitric oxide and reactive oxygen species and by indoleamine-2,3-dioxygenase mediated degradation of tryptophan. These effectors are induced not only in experimental animals, but also in the sites of immune control in infected humans. The ultimate clearance of rickettsiae is mediated by cytotoxic T lymphocyte-induced apoptotic death of the remaining infected cells. Lethal infection is associated with rickettsial antigen-specific immunosuppression mediated by induced T regulatory cells.

Future therapeutic advances that might improve survival of severely ill patients who are diagnosed late in the course, include blocking the pathogenic mechanisms such as rickettsia-induced oxidative stress and modulation of the pathologic effects of the immune response such as T regulatory cell mediated immunosuppression.
Sepsis in pregnancy

Sepsis in pregnancy is generally considered under the description of ‘puerperal sepsis’. It is one of the commonest causes of maternal death globally.

Puerperal sepsis is defined by the WHO (1995) as:

Infection of the genital tract occurring at any time between the rupture of membranes or labour, and the 42nd day postpartum in which 2 or more of the following are present:

- Pelvic pain
- Fever =>38.5 deg C
- Abnormal vaginal discharge eg pus
- Abnormal smell of discharge
- Delay in the rate of reduction of size of uterus (<2cm/day during the first 8 days)

Puerperal infection is a more generalised term, and includes extra-genital and incidental infections:

1. Infections of the genito-urinary system related to labour, delivery, and the puerperium:
   - infections related to the uterus and its associated structure (endometritis) ie puerperal sepsis (see above)
   - infections related to the urinary tract
2. Infections specifically related to the birth process but not of the GU system, eg breast abscess
3. Incidental infections eg malaria, respiratory tract infection

These case definitions are obviously written for regions that have high maternal mortality rates and poor health resources. They do not necessarily include anatomic pathology and microbiological investigations. They also – oddly – do not include HIV infection in pregnancy.

Problems

During the course of examining at autopsy many women who die during or shortly after pregnancy in south England, and reviewing hundreds of case reports as part of the UK triennial reports into maternal deaths, I have found these definitions unsatisfactory. They blur different microbiological-clinical-pathological scenarios, and thus hide opportunities for prevention and/or improved case management.

The purpose of this presentation is to open up discussion into the following areas:

1. What are the organisms causing sepsis in pregnancy?
2. How do they enter the mother, ie by which route?
3. How do they cause severe sepsis and fatality?
Taxonomically, pregnancy related sepsis is usually categorised as Direct Maternal Deaths (ie would not happen if the women was not pregnant); I think that many are actually Indirect MDs (ie conditions that can happen to any woman, but exacerbated by pregnancy.

Definitions

Case definitions for sepsis in pregnancy should include the following

- Clinical aspects
- Chronological aspects
- Microbiology
- Histopathology (sites of infection and whole body response)

Proposed new classification based on pathogenetic scenarios:

1. Following unsafe abortion – this is conceptually the most straight forward

2. Spontaneous or induced rupture of membranes with concurrent ascending genital tract infection; at any gestation; outcome of fetus irrespective - puerperal sepsis as defined by WHO (above)

3. Normal vaginal, instrumental or Caesarean delivery – interval without signs or symptoms of infection =/ >24 hours – puerperal sepsis as defined by WHO (above)

4. Intact membranes, not in labour, presenting with severe systemic sepsis - genital tract may or may not demonstrate infection - outcome of fetus irrespective - microbiology is usually Streptococcus; at any gestation

5. Other infections in pregnancy and puerperium other than the above (eg HIV-associated, breast abscess, TB, malaria, infective endocarditis)

Pathologically, each of these may be subdivided into those with histologically and/or microbiologically proven genital tract inflammation related to infection (upper vagina, cervix, endometrium, placental components), and those without, or not ascertained.

Pathogenesis

The particularly interesting scenarios are 3 & 4, and this is where the uncertainty over the route of entry arises. I have seen many #4 cases at autopsy.

In scenario #3, the infection may be related to delivery (ie Semmelweis scenario); or it could be community-acquired (inhaled) and the portal of entry into blood may be as #4 (below). In the former scenario, it is a Direct MD; in the latter, an Indirect MD.
In scenario #4, some of these patients had community-acquired sore throats (presumed or proven *Streptococcus*). Traditionally it was thought that the infection accessed the blood stream directly in the oro-pharynx to cause bacteraemic severe sepsis. But our microbiologists think it plausible that the oro-pharyngeal infection is transmitted manually to the vagina, and gains access to the blood via the genital tract. My hypothesis is thus: being pregnant makes access of this vaginal infection into the blood easier than in the non-pregnant, because the cervix is somehow different.

I am also struck by my autopsy experience of severe sepsis in previously healthy non-pregnant or post-partum young people. Excluding those with primary pneumococcal meningitis or meningococcaemia, they have either GAS or *Strep.pneumoniae* bacteraemic septic shock – and all are women (no men). Thus I suggest that of the scenario #4 maternal deaths I have seen at autopsy, all are community-acquired infections exacerbated by the state of pregnancy; ie they are Indirect and not Direct sepsis deaths.

**Question**: how much weight should be placed on histological identification of inflammation in the genital tract and/or placenta in determining whether or not an infection entered the mother via the genital tract?

**Epidemiology evidence**

Approaching UK national epidemiological data, I would therefore expect to see:
1. that strep bacteraemia is more common in women than men overall (when IVDUs are excluded)
2. within women, strep infection is more common in relation to pregnancy, ie the incidence of these infections in pregnant women in the 15-44yr age group is greater that the incidence in non-pregnant women in the same age range.

The Health Protection Agency (HPA) website gives 2007 data that, indeed, GAS is more common in women only in the 15-44yr age group. But IVDU is a major confounding factor (and those cases are mainly men, and need to be filtered out. Essentially, national streptococcal infection data is not sufficiently stratified to answer the questions.

**Conclusion**

Depending on whether this approach to sepsis classification is robust, it may alter how we write the Sepsis sections in reports on maternal death and make recommendations on remediable factors. It also introduces a new way of thinking about screening for vaginal carriage of streptococci in pregnancy.

Sebastian Lucas

Dept of Histopathology, St Thomas’ Hospital, London SE1 7EH, UK

Email: sebastian.lucas@kcl.ac.uk
Allergic bronchopulmonary aspergillosis (ABPA), first described by Hinson and colleagues in 1952, is caused by fungi in the genus *Aspergillus*. Most people with ABPA have asthma and/or cystic fibrosis, although the disorder can develop in the absence of these conditions or rarely in individuals with hyper-immunoglobulin E syndrome or chronic granulomatous disease. Most studies report the prevalence of ABPA to be 1-2% in people with persistent asthma and 2-15% in people with cystic fibrosis, but some more recent studies report a prevalence in asthma clinics as high as 20% and a prevalence of 38.6% in patients with acute severe asthma admitted to a respiratory intensive care unit. Among patients with bronchiectasis seen at one large referral center, ABPA was determined to be causative in 7.9%, the third most frequent etiology after infection (31.5%) and primary ciliary dyskinesia (10.3%). Underdiagnosis and delayed diagnosis of ABPA are common and problematic, as this condition has been associated with increased severity of asthma and progressive lung damage leading to bronchiectasis and parenchymal scarring. Fortunately, physician awareness of ABPA appears to be increasing, fostering its detection and therapy. Routine skin test screening for *Aspergillus* hypersensitivity in patients with asthma, with additional evaluation for ABPA in *Aspergillus*-hypersensitive patients, has been advocated. Particular challenges remain in diagnosing ABPA in patients with cystic fibrosis, but new clinical laboratory assays have the potential to improve diagnosis in this patient population.

Using morphological, physiological and molecular approaches to delineating taxonomy, approximately 250 species of *Aspergillus* have been recognized. The most common human pathogens are *A. fumigatus*, *A. flavus*, and *A. niger*. *A. fumigatus* causes more than 80% of cases of ABPA. Aspergilli are found throughout the world in decomposing organic matter in soil, mulches, and foods, and can be isolated in cultures from buildings where water damage has occurred. Inhalation of airborne spores begins the sequence of events leading to pulmonary infection.

Pulmonary infection is associated with a spectrum of syndromes as shown below (Table 1). Evolution from a less aggressive syndrome to a more aggressive syndrome is unusual, as are combinations of ABPA and aspergilloma or allergic *Aspergillus* sinusitis.
Table 1. Pulmonary syndromes caused by *Aspergillus* spp.

<table>
<thead>
<tr>
<th>Pulmonary Syndrome and Presentation</th>
<th>Patient Characteristics and Predisposing Factors</th>
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<tbody>
<tr>
<td><strong>Allergic bronchopulmonary aspergillosis</strong></td>
<td>Asthma or cystic fibrosis, rarely hyper–immunoglobulin E syndrome or chronic granulomatous disease</td>
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<tr>
<td>• Wheezing, expectoration of sputum containing brown plugs, pleuritic chest pain, fever</td>
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<tr>
<td><strong>Aspergilloma</strong></td>
<td>Cavitary lung disease or bronchiectasis due to tuberculosis (most common), sarcoidosis, bacterial or other fungal infections, bronchial cysts, bulla, neoplasms</td>
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<tr>
<td>• Often asymptomatic or have hemoptysis of varying severity</td>
<td></td>
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<tr>
<td>• Less commonly, cough and dyspnea secondary to underlying lung disease</td>
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<tr>
<td><strong>Hypersensitivity pneumonia</strong></td>
<td>Sensitive host</td>
</tr>
<tr>
<td>• Subacute and chronic presentations are more common than acute</td>
<td></td>
</tr>
<tr>
<td><strong>Chronic necrotizing pulmonary aspergillosis</strong></td>
<td>Chronic lung diseases including COPD, pneumoconiosis, cystic fibrosis, lung infarction, sarcoidosis, or previous tuberculosis, thoracic surgery, radiation therapy</td>
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<tr>
<td>• Weeks or months of fever, weight loss, malaise, fatigue, chronic productive cough, and hemoptysis of weeks-months duration</td>
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<tr>
<td>• Occasionally, patients are asymptomatic</td>
<td></td>
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<tr>
<td><strong>Invasive tracheobronchial aspergillosis</strong></td>
<td>Diabetes mellitus, alcoholism, chronic liver disease, low-dose corticosteroid therapy, malnutrition, and connective tissue diseases</td>
</tr>
<tr>
<td>• Cough, hemoptysis</td>
<td>AIDS, lung transplantation, other immunocompromising conditions</td>
</tr>
<tr>
<td><strong>Invasive aspergillus pneumonia</strong></td>
<td>Prolonged neutropenia (&lt;500 cells/mm³ for &gt;10 days), chronic granulomatous disease, transplantation (especially lung and HSCT), prolonged and high-dose corticosteroid therapy, hematological malignancy (especially leukemia), cytotoxic therapies, or advanced AIDS</td>
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<tr>
<td>• Symptoms are consistent with bronchopneumonia: fever unresponsive to antibiotics, cough, sputum production, dyspnea; pleuritic chest pain associated with infarcts due vascular invasion, and hemoptysis can also occur</td>
<td></td>
</tr>
<tr>
<td>• Mortality rate exceeds 50% in neutropenic patients, and 90% in hematopoietic stem-cell transplantation (HSCT) recipients</td>
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PULMONARY PATHOLOGY

In ABPA, *Aspergillus* airway colonization leads to a range of host responses involving the airways at all levels: mucoid impaction of bronchi and bronchiectasis, bronchocentric granulomatosis, bronchiolitis, and eosinophilic pneumonia. With mucoid impaction, bronchial lumens fill with “allergic mucin,” which demonstrates a characteristic lamellated pattern of cells and debris, including histologically intact and necrotic eosinophils and other cells, cellular debris, Charcot-Leyden crystals, mucus, and fungal hyphae. The fungal hyphae are found in the mucinous and cellular material, but are typically sparse and fragmented, making identification difficult. Although hyphal invasion of the bronchial wall is absent, the bronchial wall is distorted by features of asthma and bronchiectasis including a polymorphous inflammatory infiltrate of eosinophils, lymphocytes and plasma cells; thickening of the basement membrane; variable degrees of goblet cell hyperplasia, squamous metaplasia, epithelial cell injury, and ulceration; and often muscular and cartilaginous loss and mural fibrosis.

Bronchocentric granulomatosis refers to the replacement of small airway walls by necrotizing granulomas that are typically centered upon the airway lumens. Small numbers of often fragmented *Aspergillus* hyphae can be demonstrated in the centers of the necrotizing granulomas. Airway walls can demonstrate infiltration by eosinophils and lymphocytes, and lumens contain allergic mucin or show evidence of obliterative bronchiolitis. Bronchocentric granulomatosis is not unique to ABPA, but can also represent a consequence of pulmonary infection with mycobacteria, other fungal species, or echinococcus, or involvement by Wegener's granulomatosis or rheumatoid arthritis.

Eosinophilic pneumonia, characterized by alveolar infiltrates of eosinophils and macrophages, is another potential manifestation of ABPA that may occur as the sole finding or in combination with mucoid impaction or bronchocentric granulomatosis. Finally, aspergilloma can rarely occur in a setting of ABPA. Secondary post-obstructive changes of acute or organizing bacterial pneumonia, abscess formation, lipoid pneumonia, and chronic interstitial pneumonia may also accompany the primary lesions of ABPA.

PATHOGENESIS

ABPA develops in a subset of patients with hypersensitivity to the fungus. A recent meta-analysis revealed a prevalence of *Aspergillus* hypersensitivity in asthma of 28%. A variety of genetic and environmental factors have been implicated in the pathogenesis of ABPA. The genetic factors were recently reviewed by Agarwal and include presence of HLA DR-2 and absence of HLA-DQ2 sequences, CFTR gene mutations, and certain polymorphisms of the IL-10 promoter, surfactant protein A, IL-15, TNF-α, IL-4 receptor, IL-13, mannose-binding lectins and a Toll-like receptor. Most of these factors affect antigen presentation and immune responsiveness. Familial occurrence of ABPA was 4.9% in one series.

The pathogenesis of ABPA involves interacting cellular and biochemical events that lead to airway injury and fibrosis. Type I (immediate hypersensitivity), type III (antigen-antibody
complexes), and type IVb (eosinophil-rich inflammatory responses) hypersensitivity mechanisms form components of the immune responses in this condition. Inhaled conidia germinate into hyphae which become entrapped in the mucus filling asthmatic airways. Fungi cause direct damage to airway epithelial cells by releasing proteases which facilitate antigen transport across the epithelial cell layer, and interacting with airway epithelial cells to prompt release of pro-inflammatory cytokines and chemokines that initiate an immunologic/allergic inflammatory response.\textsuperscript{20,21} The fungus induces a strong Th2-type response with markedly elevated \emph{Aspergillus}-specific and total serum IgE levels and a strong eosinophilic inflammatory response.\textsuperscript{22} Epithelial cell release of growth factors is promoted by Th2-type cytokines (IL-4 and IL-13), and repair and remodeling proceed to create the bronchiectatic lesions and fibrosis characteristic of ABPA.\textsuperscript{20}

**CLINICAL, RADIOLOGIC, AND LABORATORY DIAGNOSIS**

Low-grade fevers, productive cough, and increased wheezing are common symptoms of ABPA. Patients with mucoid impaction may expectorate brown mucus plugs, providing a clue to the diagnosis as well as a stainable and culturable sample. Occasional patients are asymptomatic. ABPA often begins in childhood and can smolder for years before the condition is diagnosed.\textsuperscript{23} Most patients are diagnosed in the third or fourth decades, and there is no gender predilection.\textsuperscript{7} Radiographic findings depend upon the pathologic features present. Computed tomography (CT) frequently shows central (proximal) bronchiectasis involving primarily the segmental and subsegmental bronchi of the upper lobes.\textsuperscript{24} In asthmatic patients, bronchiectasis in three or more lobes, centrilobular nodules and mucoid impaction were found to be highly suggestive of ABPA.\textsuperscript{25} High-attenuation mucus (mucus visually denser than the paraspinal muscle) is reportedly characteristic of ABPA, but is seen in a minority of patients.\textsuperscript{26} Mucoid impaction and post-obstructive atelectasis may be apparent. In addition, peripheral infiltrates are common and may be fleeting.\textsuperscript{27} If bronchocentric granulomatosis is present, then the CT may show a focal mass or lobar consolidation with atelectasis.\textsuperscript{28}

The Rosenberg-Patterson criteria form the basis of a diagnosis of ABPA, with the presence of six of the following criteria making the diagnosis of ABPA almost certain: presence of asthma, current or previous infiltrates on chest radiograph or CT, immediate cutaneous reactivity to \emph{Aspergillus} using a skin-prick test or intradermal injection, elevated total serum IgE concentration (cutoff of 417 IU/mL is often used), serum precipitating antibodies to \emph{A. fumigatus}, central bronchiectasis, peripheral blood eosinophilia (>1.0 x 10^9/L), and elevated serum IgE and/or IgG to \emph{A. fumigatus}.\textsuperscript{6,23-29,31} Although these major criteria are generally accepted, application of the criteria varies considerably between centers. Minor criteria include finding \emph{Aspergillus} in sputum, expectoration of brown mucus plugs, and a delayed skin reaction to \emph{Aspergillus} antigen (type III).\textsuperscript{7} If a patient has central bronchiectasis, the essential criteria include asthma, immediate cutaneous reactivity to \emph{Aspergillus} antigens, and serum IgE level > 417 IU/mL. Patients without central bronchiectasis are labeled as "ABPA-seropositive" if they have asthma, immediate cutaneous reactivity to \emph{Aspergillus}, serum IgE level > 417 IU/mL, history of radiographically compatible pulmonary infiltrates, and elevated levels of serum IgE.
and IgG antibodies to *A. fumigatus*. Lung biopsy is usually not needed for diagnosis, but is occasionally important for patients with atypical clinical and laboratory features. In these patients, lung biopsy and cytology specimens showing "allergic" mucin and fungal hyphae can offer confirmation.

A staging system has been applied to patients with asthma and ABPA, but is not often used for patients with ABPA and cystic fibrosis. Five stages of ABPA were described: acute, remission, exacerbation, corticosteroid-dependent ABPA, and fibrotic (end-stage) ABPA.

In a setting of cystic fibrosis, the diagnosis of ABPA is often not straightforward due to overlap of many of the diagnostic criteria with common clinical and radiologic manifestations of cystic fibrosis. Although *A. fumigatus* is common (prevalence of 10-60%) in the sputum of cystic fibrosis patients, mere presence is not associated with worsening of lung function if sensitization has not occurred. However, ABPA should be suspected in patients with cystic fibrosis who fail to improve with antibiotic therapy for an exacerbation of cystic fibrosis. Proposed minimal criteria for diagnosis of ABPA in cystic fibrosis include acute or subacute clinical deterioration not attributable to another etiology, total serum IgE concentration of > 417 IU/mL, immediate cutaneous reactivity to *Aspergillus* or presence of serum IgE to *A. fumigatus*, and one of the following: precipitins to *A. fumigatus* or IgG to *A. fumigatus*, or new or recent abnormalities on chest radiography (infiltrates or mucus plugging) or chest CT (bronchiectasis) that have not cleared with antibiotics and standard physiotherapy. Distinction of ABPA from infection-related exacerbations in cystic fibrosis can be very difficult. Serologic testing for the Th2 chemokine thymus- and activation-regulated chemokine (TARC) appears promising for identifying and monitoring ABPA in patients with cystic fibrosis. In one study, diagnostic accuracy was greater for TARC (93%) than for total IgE (74%), or specific IgE against recombinant *A. fumigatus* allergens (rAsp f) 1, 3, 4 and 6. Serological measurement of antibodies against recombinant *A. fumigatus* antigens, however, continues to undergo evaluation as a laboratory tool for diagnosis of ABPA, and appears to have potential for determining *Aspergillus* sensitization in patients with cystic fibrosis. Antibody titers against the 18-kDa ribonuclease (RNU), the 360-kDa catalase (CAT), and the 88-kDa dipeptidylpeptidase V (DPPV) of *Aspergillus* have also been studied as diagnostic markers and in one study were found to be useful for the diagnosis of ABPA in patients with and without cystic fibrosis. Serum levels of CCL17 have also been reported to be useful for identifying ABPA in cystic fibrosis and asthma patients. The cellular allergen stimulation test (CAST), which measures cysteinyl-leukotrienes produced by allergen-stimulated basophils in vitro appears to have high (100%) specificity, but a lower specificity (74%), and may be useful in combination with total serum IgE and studies of IgE antibodies to recombinant *Aspergillus* antigens in patients with ABPA. Additional large, multicenter trials are needed to determine the utility of these laboratory methods for diagnosis and monitoring of ABPA in patients with cystic fibrosis.

**MANAGEMENT**

Therapy for ABPA is geared to reduce inflammation and immunologic activity (oral corticosteroids) and decrease fungal colonization of airways (oral itraconazole).
Corticosteroids are viewed as the mainstay of therapy; specific regimens are determined based on stage and treatment responsiveness. Total serum IgE level can be followed as a marker of immunologic activity and treatment effectiveness. A 35-50% decline after six weeks-three months of corticosteroid therapy has traditionally been viewed as indicating remission of the ABPA, but does not always predict clinical responsiveness. Limited investigations of itraconazole showed improved clinical outcomes, reduction of IgE, and reduction of daily corticosteroid dose, but lung function did not significantly improve. Nonetheless, itraconazole has been recommended by the Infectious Diseases Society of America as a component of ABPA treatment and is used as adjuvant therapy in many centers. Avoidance of areas in which the fungus grows particularly well (moist basements, crawl spaces, compost piles, and others) may be beneficial, although the widespread distribution of the fungus in the environment does not allow for avoidance of exposure. Treatment of underlying conditions (asthma, cystic fibrosis) is also naturally recommended. Finally, in a small number of patients with ABPA and cystic fibrosis, anti-IgE (omalizumab) antibody has yielded good results with improvement in pulmonary symptoms and lung function, as well as discontinuation of systemic corticosteroids in some.

**DIFFERENTIAL DIAGNOSIS**

Although *A. fumigatus* is the most common *Aspergillus* species to trigger ABPA, other *Aspergillus* species including *A. niger*, *A. flavus*, *A. nidulans*, *A. oryzae*, and *A. terreus* are occasionally responsible, and a role for multiple species can also occasionally be demonstrated. If cultures, skin tests, and antibody assays for *Aspergillus* are negative in the face of clinical and radiographic features suggestive of the disease, then a fungus other than *Aspergillus* may be responsible. Similar syndromes can be caused by other fungi ("allergic bronchopulmonary fungal disease" or "allergic bronchopulmonary mycosis"), including *Candida albicans*, *Curvularia sp.*, *Helminthosporium sp.*, *Torulopsis glabrata*, *Bipolaris sp.*, *Cladosporiosis sp.*, *Saccharomyces cerevisiae*, *Schizophyllum commune*, and *Tricosporon beigeli*, and asthma is not invariably present in these patients. Other entities in the differential diagnosis of ABPA include asthma with *Aspergillus* hypersensitivity but without ABPA, pulmonary infections with tuberculosis and non-tuberculous mycobacteria, Churg-Strauss syndrome, and bronchiectasis, eosinophilic pneumonia or bronchocentric granulomatosis associated with other etiologies or as idiopathic conditions.
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