

Editorial

Comprehensive diagnostics for respiratory virus infections after transplantation or after potential exposure to swine flu A/H1N1: what else is out there?

Virus infections of the respiratory tract have received significant public attention in the last decade through the SARS epidemic in 2003, the increasing pandemic threat of avian influenza H5N1 since 2005, and now the unexpected striking of influenza A/H1N1 in 2009 (erroneously called 'swine flu' in North America and Europe). As happened with the Spanish Pandemic of 1918, the emergence of previously unknown viruses such as SARS has catalyzed important progress in clinical virology: 1) Development and stringent application of case definitions and infection control measures to limit transmission and eventually contain the spread in disease; 2) International communication and collaboration for the speedy identification and characterization of the implicated infectious agent; 3) Introduction of rapid nucleic acid testing (NAT) of high sensitivity and specificity with a turn-around time of < 24 h for clinical management; and 4) Freely accessible information flow through specific websites on all of these issues.

These points effectively synergized during the recent emergence of the new 'swine flu' influenza A/H1N1 and appeared to permit, under close surveillance, a seemingly attenuated entry into a new influenza pandemic after more than 30 years. Although the overall clinical course of the 'swine flu' appears unexpectedly mild, mostly younger healthy adults between 25 and 45 years of age seem to be affected, similar to the Spanish H1N1 pandemic of 1918.

However, it is still way too early to give the 'all-clear':

- First, younger adults are probably the most active traveling population and simply the first to be exposed to the virus, thus carrying the pandemic around the world and home again. This population is also more likely to have young children, causing secondary clusters in kindergarten and schools and hence refueling the pandemic with their higher and prolonged shedding.
- Second, early identification and treatment with neuraminidase inhibitors may have significantly attenuated viral replication and cytopathology and hence the clinical presentation, while, with an accelerating spread of the pandemic, delayed and deferred treatment may allow more severe clinical courses. This scenario may par-

ticularly apply to countries of the developing world where access to health care and neuraminidase inhibitors is limited, but co-morbidities are abundant.

- Third, the current human-to-human transmission of the new influenza A/H1N1 is significantly more efficient compared with SARS and the avian H5N1, and spreads in the northern hemisphere outside of the cold season. Patients suffering from SARS became infectious at the onset of clinical disease; however, influenza cases are highly infectious 1–2 days *before* becoming symptomatic. As the cold season is just starting in the southern hemisphere with the seasonal influenza epidemic, double the number of infections can be anticipated in humans, who will become mixing vessels for new, more pathogenic variants.
- Fourth, emergence of neuraminidase inhibitor-resistant variants is looming similar to the influenza season 2007–2008 (1).

Thus, as the burden of the new influenza pandemic is yet to be defined in this early stage, laboratory diagnostics are critical for unambiguous diagnosis and appropriate management.

As early as in the first week of May 2009, we provided a 7-days-a-week service for the University Hospital Basel and the northwestern region of Switzerland, by establishing polymerase chain reaction (PCR)-based diagnostic NAT targeting specifically only the hemagglutinin gene of the new influenza A/H1N1 by adapting the oligonucleotide sequences described by Richt et al. (2) (SIV.H1-F: AACAAATCAACAGACACTG, SIV.H1-R: GTTTGCATAGTTTC-CCGT, SIV.H1-P: AAGAATGTAACAGTAACACACTCTG). Among the 86 suspect cases tested until June 22, 2009, we identified the new influenza A/H1N1 in 7 patients (8.1%). The median viral load of the positive samples was 2.5×10^5 copies/mL, but the interquartile range was broad, reaching from 1.07×10^5 to 5.97×10^6 copies/mL. All of the 7 confirmed cases had recently traveled from the USA. All 7 were treated with oseltamivir 75 mg twice a day and recovered without significant complications.

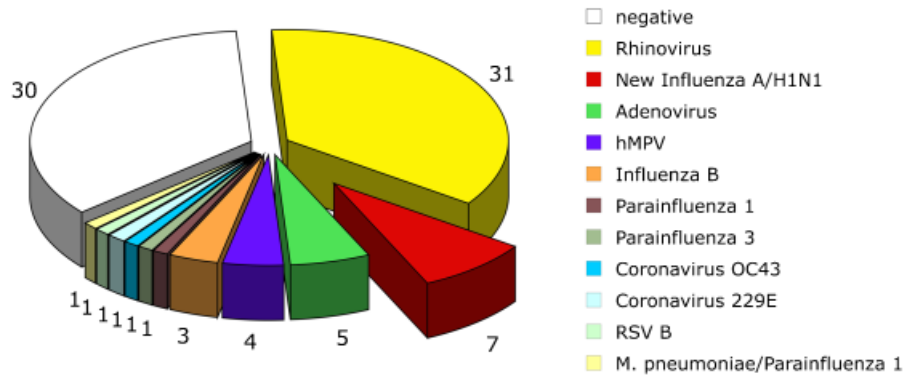


Fig. 1. Results of nucleic acid testing multiplex assay for 86 cases with suspected swine flu in Switzerland. hMPV, human metapneumovirus; RSV, respiratory syncytial virus; M., *Mycoplasma*.

As we had a multiplex NAT in place that could identify 19 respiratory pathogens (3), we wondered what else was present in the remaining 79 individuals. As shown in Figure 1, respiratory viruses were detected in 56 (65%) of the patients, with the leading pathogen being rhinoviruses (RhV) in 31 (36%), followed by adenovirus (ADV) in 5 (6%), human metapneumovirus (hMPV) in 4 (5%), influenza virus B (FLU-B) in 3 (3%), coronaviruses (CoV, 2%), and parainfluenza (PIV, 2%) in 2 cases each, as well as 1 dual infection with PIV-1 and *Mycoplasma pneumoniae*. Respiratory syncytial virus (RSV)-B was detected in 1 sample (1%). No pathogen was detected in 35% of our patients, suggesting a role for still other pathogens or possibly non-infectious causes including sampling errors. However, sampling errors are not very likely because the strategy was well defined (1 nasal swab plus 1 throat swab both into viral transport medium) and performed under dedicated isolation precautions.

Nevertheless, our overall detection rate of 65% outside of the cold season is strikingly higher than the approximately 30% reported for conventional assays or even other studies using comprehensive NAT for respiratory virus infections (4). In the large single-center cohort study by Garbino et al. (5), NAT identified viruses in 91 (17%) of 522 bronchoalveolar lavage fluids. Two-thirds of the samples were obtained from lung transplant and other transplant patients with the leading agent being CoV (32%), followed by RhV (23%), PIV (20%), and FLU (10%) (5).

The increased vulnerability to novel respiratory viruses is caused in part by the lack of specific antiviral immune memory responses, which otherwise reduce the clinical disease by decreasing the viral load through neutralization of infectious particles, and effective killing of infected host cells. While this vulnerability naturally applies to small children, transplant patients are similarly at risk for higher and longer virus replication, increased virus-mediated

cytopathology, and progression to more extensive inflammation and organ dysfunction. Although the intensity of immunosuppression is a key determinant, the allogenic constellation between the virus-infected cells and the cellular immune effectors contributes to an impaired antiviral immune control. Accordingly, morbidity and mortality of respiratory virus infections is accentuated after lung or hematopoietic stem cell transplantation (6). Moreover, longer term consequences of decreased pulmonary function, bronchiolitis obliterans, and related syndromes become more likely.

As a wide variety of viruses circulates in the community with overlapping seasonality and clinically largely indistinguishable symptoms and signs, their impact in transplant recipients is only incompletely understood. In the single-season cohort study of 388 adult lung transplant patients, Gottlieb et al. (7) identified 34 community-acquired respiratory viruses in 30 patients (7.7%) including PIV in 12 (35%), RSV in 7 (20%), hMPV in 6 (18%), and CoV in 5 (15%). The 1-year incidence of bronchiolitis obliterans syndrome was 25% in virus-positive patients, significantly higher than the 9% observed in virus-negative patients (7).

Two reports in this issue of *Transplant Infectious Disease* further highlight the importance of respiratory pathogens in the context of transplantation. The retrospective multicenter study by Liu et al. (8) analyzed risk factors and outcome of respiratory viral infections in 576 pediatric patients within the first year after lung transplantation. In this high-risk population (i.e., being pediatric, immunosuppressed, and lung-transplanted), 101 respiratory virus infections were reported in 79 patients (14%). By mostly non-molecular methods, a wide variety of viruses was detected in the patients showing respiratory symptoms, the most common being ADV (25%), RhV (22%), RSV (21%), and PIV (19%). Importantly, detection of respiratory virus infections was more frequent in younger children and was

independently associated with a decreased 1-year survival (8). As stated by the authors, the usage of modern NAT, which was not readily available in the study period from 1998 to 2005, might have increased the diagnostic yield. NAT, especially real-time PCR assays, nowadays plays a central role in virus diagnostics and is increasingly considered as the 'gold standard' for the detection of viruses.

The article by Kuypers et al. (9) in this issue of *Transplant Infectious Disease* illustrates this shift to molecular diagnostics as the reference method for respiratory infections in hematopoietic cell transplant recipients. By using PCR assays targeting RSV, FLU-A and -B, PIV-1 and -3, and hMPV, the authors have obtained 2 times more positive samples than by culture alone, and 4 times more than by direct fluorescent antibody staining. A positive result by conventional diagnostic assays required significantly higher genome viral loads above 5.75 log genome equivalents (gEq)/mL in real-time reverse-transcription PCR assays. Although only 34 samples with 6 different viruses were tested (19 PIV, 6 RSV, and 3 FLU), the overall findings of Kuypers et al. (9) support the results of 2 previous studies demonstrating the superior sensitivity of PCR compared with classical methods for 34 RSV and 21 influenza cases after hematopoietic stem cell transplantation (10, 11). For the influenza cases, the threshold for positive culture results was $>5.1 \log \text{gEq/mL}$ (11), and for RSV it was $>5.3 \log \text{gEq/mL}$ (10). In another study of hematopoietic stem cell transplant recipients, PIV and RSV were associated with a significant decline in airflow (12).

Thus, we are currently witnessing a quantum leap in clinical virology where specific and sensitive NAT of respiratory viruses with a short turn-around time is qualifying as the new 'gold standard' for timely initiation of infection control and antiviral treatment, as well as for follow up (10, 11).

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