Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 1, 2012

Research Article

DIAGNOSTIC CONSIDERATIONS FOR NOVEL INFLUENZA A (H1N1)

MARWAN SHEIKH-TAHA, *EDWARD H. EILAND, III., JIAN HAN, WILLIAM LINDGREN, THOMAS MACANDREW ENGLISH, ALI HASSOUN.

*Clinical Practice and Business Supervisor, Department of Pharmacy, Huntsville Hospital. Email: edward.eiland@hhsys.org

Received: 16 Aug 2011, Revised and Accepted: 27 Sep 2011

ABSTRACT

Background: The emergence of novel swine-origin influenza A (H1N1) virus (S-OIV) infection represents a significant pandemic threat. Rapid and accurate diagnosis will enhance treatment and containment efforts.

Methods: Ninety eight patients with influenza-like illness meeting the CDC's guidelines for screening were tested with the rapid influenza test. Positive samples for influenza A were tested with real-time PCR (RT-PCR). Subsequently, all samples were tested using Target Enriched Multiplex Polymerase Chain Reaction (Tem-PCR).

Results: Rapid influenza tests confirmed 30 influenza A cases, 1 of influenza B, and resulted 67 negatives. The 30 samples positive for influenza A were tested using RT-PCR assay, which revealed 2 cases of seasonal influenza A, 7 cases of S-OIV, and 21 negatives for influenza infection. The Tem-PCR confirmed 1 of the seasonal influenza A cases but found the second case to be S-OIV. Tem-PCR confirmed the findings of RT-PCR in 3 of the S-OIV cases but reported 4 of the cases negative for influenza. Additionally, Tem-PCR found 3 of the cases that were negative per RT-PCR were positive for S-OIV. The remainders were confirmed negative for influenza yet one was positive for adenovirus. The lone case of influenza B was confirmed with Tem-PCR. Among the 67 cases that were negative based on rapid influenza tests, Tem-PCR confirmed one case positive for S-OIV, however, the remainder were influenza negative. Tem-PCR identified viral organisms that comparator tests could not.

Conclusion: Clinical judgment should be applied when interpreting the results of the available S-OIV tests. A confirmatory and validated test for S-OIV is urgently needed.

Keywords: Swine flu, Novel influenza A (H1N1), RT-PCR, Tem-PCR

INTRODUCTION

As the 2009 new swine-origin influenza A (H1N1) virus (S-OIV) emerged initially in Mexico and subsequently around the world [1], there were several challenges, including mass presentation of concerned patients to the emergency room and physicians offices with upper respiratory tract symptoms. Cases of swine flu were deemed possible with positive influenza antigen testing and suggestive symptoms as defined by the CDC, but confirmation of cases took at least 7-10 days in view of lack of access to a statewide rapid confirmatory test. Since the outbreak was first detected, an increasing number of states have reported cases of S-OIV infection with associated hospitalizations and deaths. Resurgence of the novel H1N1 disease in the fall with the potential of influenza pandemic is a possibility that we need to be prepared for. This is particularly true if new mutations increase its virulence, as occurred in 1918 when the Spanish flu pandemic, the most severe influenza pandemic known, acquired severe virulence during the second wave of human disease in the winter season [2]. To enable a quick response to a potential outbreak, it is imperative to have a rapid and accurate diagnostic method capable of discriminating novel H1N1 virus from other strains which will expedite appropriate antiviral therapy and enhance control and containment efforts.

We report the results of 3 different diagnostic tests: rapid antigen influenza, real-time reverse-transcriptase PCR (RT-PCR), and Tem-PCR (target enriched multiplex PCR), in patients with influenza-like illness who met the CDC's guidelines for screening.

MATERIALS AND METHODS

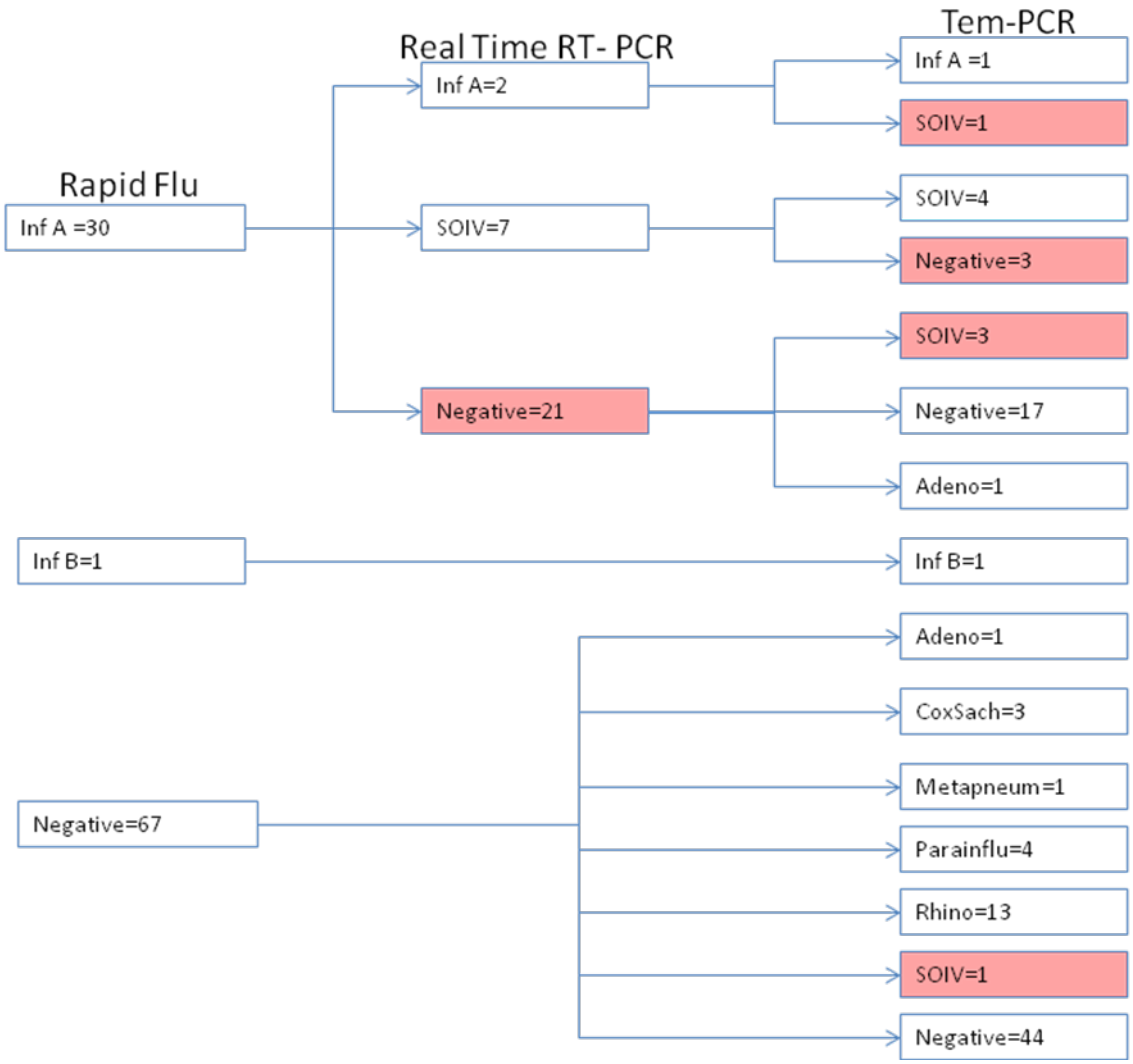
From April 27, 2009 thru May 7, 2009, nasopharyngeal swabs were collected from 98 patients who met CDC screening guidelines and presented with influenza-like illness to the emergency room at Huntsville Hospital, an 881 bed regional referral center located in North Alabama. Patients were tested using a rapid antigen influenza test, the Inverness BinaxNOW flu kit. All positive samples were tested by the Alabama Department of Health (DOH) using Roche Magna Pure LC and Compact (2 of each) for extraction and the ABI 7500 Fast for the RT-PCR assay. If the virus was non-typable, the sample was sent to the CDC for confirmation of S-OIV. Additionally, all negative and positive samples were tested using Tem-PCR looking for multiple viruses, including S-OIV, as described below. Tem-PCR is a new multiplex PCR technology called Templex, developed by Diatherix (Huntsville, AL) scientists, which utilizes the Diatherix proprietary Tem-PCR method. As with Templex technology, multiple molecular targets can be detected. The panel detects and differentiates targets specific for several upper respiratory viral pathogens, including influenza A, influenza B and S-OIV. Specifically, guidelines were followed to design the novel H1N1-09 specific primers and probes. Genomic sequences were first obtained from the Global Initiative on Sharing Avian Influenza Data (GISAID) website and aligned with other influenza A sequences. These sequences were later confirmed with those published at the GenBank to identify unique regions. A total of 4 amplification targets for nested PCR primer sets were designed and H1N1-09 specific detection probes were constructed to be used with Luminex beads array. Lastly, "mini-genes" (synthetic DNA including all primers and probe binding sequences) were synthesized to be used as templates for initial evaluation of the primer and probe performance. Moreover, Tem-PCR has the particular advantage of detecting several viruses from the same sample including adenovirus types 3, 4, 7, 21, coxsackie viruses, echoviruses, human metapneumovirus, human influenza A and B, parainfluenza virus types 1 through 4, respiratory syncytial virus A& B, and rhinoviruses. The test requires only one patient sample and only one reverse-transcription PCR reaction. At the time of writing the manuscript, the FDA was evaluating an Emergency Use Authorizations (EUA) for the Tem-PCR test for the diagnosis of S-OIV.

RESULTS

Rapid influenza antigen tests revealed 30 specimens positive for influenza A, one positive for influenza B, and 67 that were negative. The 30 samples that were positive for influenza A were tested by the

DOH and CDC with RT-PCR assay, which revealed two cases of seasonal influenza A, seven confirmed cases of S-OIV, and 21 remaining samples that were negative for influenza infection. The Tem-PCR confirmed one of the seasonal influenza A cases but found the second case to be S-OIV. Tem-PCR confirmed the findings of RT-PCR in three of the S-OIV cases but found that the other four cases were negative for influenza. Additionally, Tem-PCR found that three of the cases ruled negative based on RT-PCR were actually positive for S-OIV. The remaining samples were confirmed negative for influenza but one was positive for adenovirus. The lone case of influenza B was confirmed with Tem-PCR. The 67 cases that were negative based on rapid strep results were also tested using Tem-PCR. Tem-PCR found that one case was positive for S-OIV but the rest of the cases were negative for influenza. Tem-PCR found several other organisms that the other tests could not detect. It found one case of adenovirus, three cases of coxsackievirus, one case of metapneumovirus, four cases of parainfluenzavirus, and 13 cases of rhinovirus (Table 1).

Table 1: Tree Diagram of Diagnostic Testing Methodologies Utilized



DISCUSSION

Our study evaluated the performance of three diagnostic tests in patients with influenza-like illness who met CDC screening guidelines. When comparing the rapid influenza tests to Tem-PCR in our study, we found the rapid tests to have a sensitivity and specificity of 90% and 76%, respectively, with a positive predictive value of 30% and a negative predictive value of 99%. Our results differ from the findings of Faix et al, who reported that the rapid influenza tests have a sensitivity and specificity of 51% and 99%, respectively, when detecting S-OIV [3]. Our analysis used a genotypic test to assess the accuracy of rapid influenza testing, which may have allowed more direct verification of rapid test results.

RT-PCR is considered the gold standard for detection of influenza viruses due to its high assay specificity, sensitivity and broad linear dynamic range. After a public health emergency had been declared by the Secretary of Health and Human Services, the FDA authorized RT-PCR Swine Flu Panel diagnostic test to be a preferred diagnostic molecular test, based on EUA authority. This resulted from the fact that RT-PCR may be effective in testing samples from individuals diagnosed with influenza A infections, whose virus subtypes cannot be identified by currently available phenotypic testing methods. A positive result from the RT-PCR indicates that the patient is "presumptively" infected with swine flu virus. However, a negative result does not, by itself, exclude the possibility of a swine flu virus infection being present [4]. This was confirmed by our data.

The differences between RT-PCR and Tem-PCR results in our study could be attributed to a difference in target genes, sequence variation in primer or probe targets, and human errors, as well as other causes. With RT-PCR, false negative results may occur due to sequence variation in primer and probe targets. With Tem-PCR this is less likely to happen because multiple targets are used, and this may serve as a means of confirming positive results going forward. Additionally, this may explain why three patients tested negative for S-OIV with RT-PCR and positive with Tem-PCR. Another advantage of Tem-PCR test is that it is a more rapid test capable of identifying other micro-organisms that the RT-PCR result will not provide.

The result of Tem-PCR in our study was available within 6 hours. The identification of other viruses showed that the clinical criteria for diagnosing this emerging infection are helpful but not specific for swine flu infection. Beyond serving as a confirmatory test for S-OIV, Tem-PCR can detect other viral infections, which may aid in reducing further measures recommended in cases of swine flu including treatment of patient contacts, isolation of patients leading to the loss of workdays, unnecessary treatment, and possible lessening or avoidance of developing resistance to available antiviral pharmacotherapy.

Vasoo et al [5] described the low to moderate sensitivity of 3 rapid influenza antigen tests for novel H1N1 virus. Most notably, the authors reported specificity, positive predictive value (PPV), and negative predictive value (NPV). The estimations for these will be biased because no samples that were negative for respiratory viruses were included. This should be considered because the volume of patients that need testing may increase, especially with the manner in which the media is portraying H1N1. One of the limitations of Vasoo et al's study, which was partially addressed by the authors, is the inclusion of specimens that were only positive for respiratory viruses, using PCR assay, which can accurately estimate sensitivity but not specificity, PPV, or NPV. Thus prevalence was adjusted, which is optimal, yet specificity was not. This makes the diagnostic tests look as accurate as possible but does not portray how poor the tests could potentially perform when accurately identifying the novel H1N1 virus. Assuming the specificity is 86.2%, which is at the bottom of their confidence interval, the PPV would only be 42% for the BD test. In addition, one would disagree with the authors' statement that the rapid tests are useful even with sensitivities as low as 38% where testing would tell 62% of people with novel H1N1 that they do not have the virus.

A well-validated, rapid, sensitive, and specific test for confirming S-OIV is urgently needed for enhancing treatment and containment efforts. Viral culture, the current gold standard for typing and sub-typing of influenza viruses, usually requires 3 to 7 days for culture of the virus. As a result, clinicians will continue to use available diagnostic tests as part of their evaluation of patients with influenza signs and symptoms, despite the fact that rapid influenza test results demonstrate a wide range of sensitivities for detection of influenza viruses [3,5,6]. We conclude that results of rapid testing should be interpreted with caution, and clinical judgment should be used when deciding whether to treat patients with antiviral therapy and in making isolation decisions.

REFERENCES

1. Dawood FS, Jain S, Finelli L, Shaw MW, Lindstrom S, Garten RJ, et. al. Emergence of a Novel Swine-Origin Influenza A (H1N1) Virus in Humans. Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team. N Engl J Med. 2009 Jun 18;360(25):2667-8.
2. Taubenberger JK, Morens DM. 1918 Influenza: the mother of all pandemics. Emerg Infect Dis. *2006* Jan;12(1):15-22.
3. Faix DJ, Sherman SS, Waterman SH. Rapid-Test Sensitivity for Novel Swine-Origin Influenza A (H1N1) Virus in Humans. N Engl J Med. 2009 Jun 29.
4. www.cdc.gov/h1n1flu/EUA/pdf/tamiflu-hcp.pdf
5. Vasoo S, Stevens J, and Singh K. Rapid Antigen Tests for Diagnosis of Pandemic (Swine) Influenza A/H1N1. CID 2009;49(7):1090-3.
6. Ginocchio CC, Zhang F, Manji R, Arora S, Bornfreund M, Falk L, et al. Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak. J Clin Virol. 2009; 45:191–5.