News Release

FOR RELEASE May 17, 2010, 8:15 a.m. CDT

FOR MORE INFORMATION, CONTACT:
Keely Savoie or Brian Kell
ksavoie@thoracic.org or bkell@thoracic.org
ATS Office 212-315-8620 or 212-315-6442 (until May 14)
Cell phones 917-860-5814 or 516-305-9251
ATS Press Room: 504-670-6926 (May 15 to 20)

Poster session time: 8:15- 10:45 a.m. May 17
Location: CC-Room 293-294 (Second Level), Morial Convention Center

DFA Unreliable in H1N1 Testing in Critically Ill Patients

ATS 2010, NEW ORLEANS— Direct Immunofluorescence Assay (DFA) testing for H1N1 influenza ("swine flu") is unreliable in ICU patients, according to a new study from Stanford University. Multiple methods exist for diagnosing influenza, but data on the utility and accuracy of these tests for H1N1 are still emerging, given the relatively recent onset of the epidemic.

"Our findings suggest that in patients with severe H1N1 influenza, in whom rapid and precise diagnosis would be most important, DFA unfortunately does not perform well. This is in contrast to less severely ill patients, where DFA appears to be quite reliable." said Chanu Rhee, M.D., a physician at Stanford University School of Medicine and lead author of the study.

The results will be presented at the ATS 2010 International Conference in New Orleans.

While PCR testing has emerged as the most sensitive and specific test for diagnosis of H1N1 influenza, availability of the test and turn-around time often limit its clinical usefulness. DFA testing is used at many institutions as an accurate and rapid means of diagnosing influenza. DFA for influenza uses a fluorescent dye attached to antibodies

that bind to flu particles. If influenza is present, the antibodies will bind to viral antigens and a bright glow can be seen in the sample using a special microscope.

Several months after the H1N1 pandemic began, Dr. Rhee and colleagues at Stanford University noticed a trend at their institution that critically ill patients with H1N1 influenza more commonly had negative DFA results than those who were less severely ill. To further investigate this observation, they reviewed the records of all patients who were admitted to the Stanford University Hospital between May 20, 2009 and January 30, 2010 with H1N1 influenza. All patients were confirmed for H1N1 influenza through either PCR or viral culture, and underwent DFA testing on a respiratory tract sample. During the research period, 19 patients were admitted to the ICU; 11 required mechanical ventilation and six died of respiratory failure.

To their surprise, Dr. Rhee and colleagues found that while DFA was a fairly accurate tool for diagnosing H1N1 in non-critical cases, it was not at all accurate for patients in the ICU. Just five of the 19 ICU patients (26 percent) had positive DFAs for H1N1 infection (four by nasopharyngeal swab, one by bronchoalveolar lavage), whereas 27 out of 33 non-ICU patients (82 percent) had a positive DFA test. The median time to first DFA was seven days in the ICU patients and three days in the non-ICU patients. Of the 31 respiratory tract samples in the ICU patients that were positive as determined by PCR, only 10 were concomitantly positive by DFA.

“For the non-ICU patients, the sensitivity of DFA was fairly good and correlated with previously published values. However, we found DFA to be significantly less sensitive in critically ill patients—those with severe respiratory distress requiring mechanical ventilation or a high degree of respiratory support in an ICU setting,” said Dr. Rhee. “Interestingly, none of the DFA samples taken from the 18 endotracheal aspirates (secretions taken from the breathing tube on patients on a mechanical ventilator) were positive, despite the presence of virus detected by PCR or by bronchoalveolar lavage.”

Dr. Rhee and colleagues were surprised by their findings, as they expected that severely ill patients would have a higher burden of viral disease, leading to easier detection. “We would have also expected that samples taken from endotracheal aspirates, where the secretions are coming from lower down the respiratory tract, would have a higher likelihood of being positive, but this was not the case,” said Dr. Rhee.

One possible explanation for the poor performance of DFA in ICU patients is that it is an over-exuberant host inflammatory response, rather than high viral load, that is responsible for severe disease. However, it remains unclear why certain patients develop severe respiratory failure from H1N1 while others with similar risk factors develop only mild symptoms.

If confirmed by further research, these findings have important ramifications. “This study reinforces the fact that patients with suspected H1N1 influenza who are severely ill should be placed in respiratory isolation and receive antiviral treatment without delay, even if DFA testing is negative” said Dr. Rhee. “This includes patients with a negative

DFA from lower respiratory tract samples. Furthermore, all critically ill patients with suspected H1N1 should have PCR testing done to confirm the diagnosis, as PCR is significantly more sensitive than DFA, though not perfect either."

"The next logical step would be analyzing data from a much larger pool of patients from different institutions to confirm these findings," said Dr. Rhee.

###

"Difficulty in Rapid Diagnosis of Novel Influenza A (H1N1) Virus Using Direct Fluorescent Antibody Testing (DFA) in Critically Versus Non-Critically Ill Patients" (Session B25, Monday, May 17, 8:15-10:45 a.m., CC-Room 293-294 (Second Level), Morial Convention Center; Abstract 2477)

**Please note that numbers in this release may differ slightly from those in the abstract. Many of these investigations are ongoing; the release represents the most up-to-date data available at press time.*

Difficulty in Rapid Diagnosis of Novel Influenza A (H1N1) Virus using Direct Fluorescent Antibody Testing (DFA) in Critically versus Non-critically Ill Patients

C. Rhee, J.H. Kim, J. Schaenman, B. Pinsky, S. Madison, D. Johnson, P. Rutherford, T. Schaffner, L.S. Tompkins, J.M. Brown
Stanford University - Stanford, CA/US

Given the low sensitivity of rapid assays for H1N1, many centers rely on DFA for the diagnosis of seasonal and H1N1 influenza. However, the reported sensitivity of DFA reports in diagnosing H1N1 varies from 47-93% and its utility in different patient subgroups has not been analyzed. In order to compare the utility of the DFA testing in patients who were critically ill (ICU) versus those who were not (non-ICU), we reviewed the records of all patients who were admitted to Stanford University Medical Center with a respiratory illness prompting a respiratory tract sample for DFA testing from May 20 through October 24, 2009. All cases were confirmed by positive rRT-PCR and/or viral culture for influenza. ICU patients were defined as those who required either mechanical or non-invasive positive pressure ventilation in an intensive care unit. Positive DFA specimens from non-ICU and all specimens from ICU patients were sent for molecular analysis to county, state, or reference laboratories.

In 29 patients hospitalized with confirmed H1N1, 10 were admitted to the ICU. The median age was 40 and 44 years in ICU and non-ICU, respectively. Concomitant conditions in the ICU patients included transplantation (N=2), pregnancy (N=3), asthma (N=3), CHF, multiple myeloma, cystic fibrosis, obesity (BMI>30, N=4). All ICU patients had radiographic abnormalities compared to only 4 non-ICU patients. Median duration of symptoms (range) prior to DFA was 2 (0-9) and 6 (1-20) days in the non-ICU and ICU patients, respectively. Only two of ten ICU patients (20%) had a positive DFA in the absence of antiviral therapy, in contrast to 17 of 19 non-ICU patients (89%). None of the endotracheal samples were DFA positive despite the presence of virus confirmed by PCR in these samples or from bronchoalveolar lavage fluid.

Although the H1N1 virus has been shown to have a greater tropism for the lower respiratory tract than seasonal flu in animal models, this would not explain our observation that patients with more severe disease paradoxically appear to have a lower viral load in the upper respiratory tract. Our observations are consistent with the possibility that severe lower respiratory tract disease in the adult, non-elderly patient may reflect an exuberant host reaction manifesting as sepsis and/or severe pneumonitis. In our early experience, DFA has been substantially less sensitive in the diagnosis of disease in critically ill versus non-ICU patients which may further delay establishing the diagnosis and instituting treatment of H1N1.

Table 1A

Non-ICU Patients: Diagnostic test results, Co-morbidities, Treatment, and Outcome

Case	Sex, Age	Day from Onset of Symptoms	DFA	RT-PCR[a]	Co-morbidities	Treatment	Outcome
1	M, 44	2	+	+[b]	AML	OSEL, ZAN	Alive
2	M, 35	1	+	+[b]	Schizoaffective	None	Alive
3	F, 64	4	+	+[b]	Asthma	OSEL	Alive
4	M, 51	1	+	+[b]	Insulinoma	None	Alive
5	F, 74	1	+	+[b]	ILD, COPD, CHF	OSEL	Alive
6	F, 18	1	+	+[b]	Asthma, Pregnancy, Obesity	OSEL	Alive
7	M, 37	4	+	+[b]	Cardiac Transplant	OSEL	Alive
8	M, 58	3	+	+[b]	Lymphoma, HCT, Obesity	ZAN	Alive
9	M, 20	1	+	+[b]	Cerebral palsy	OSEL	Alive
10	M, 20	7	-	ND	Diaphragm rupture	OSEL	Alive
		9	ND	+[c]			
11	F, 29	3	+	+[b]	Cystic fibrosis, double lung transplant	OSEL, ZAN	Alive
12	M, 50	1	+	+[c]	Rheumatoid Arthritis, DM	OSEL	Alive
13	F, 41	2	+	+[b]	DM, Obesity	OSEL	Alive
14	M, 57	3	+	+[b]	Cardiac Transplant	OSEL	Alive
15	M, 49	0	+	+[b]	ESRD, Amyloidosis	OSEL	Alive
16	F, 40	6	-	+[c]	Obesity	OSEL	Alive
17	F, 52	3	+	+[b]	COPD, Sjogren's Syndrome	OSEL	Alive
18	F, 55	2	+	+[b]	CHF, Lymphoma, DM, Hepatitis C	OSEL	Alive
19	M, 18	2	+	+[b]	Cardiac Transplant	OSEL	Alive

Table 1B

ICU Patients: Diagnostic Test Results, Co-morbidities, Treatment, and Outcome

Case	Sex, Age	Day from Onset of Symptoms	DFA for Influenza A		RT-PCR[a]	Viral Cx[d]	Co-morbidities	Tx	Outcome
			Source	Result					
1	F,50	2	NP	-	-[c]	ND	Myelofibrosis, HCT, Asthma, Obesity	OSEL, ZAN	Expired
		11	NP	+	+[b,e]	ND			
		15	ETA	-	ND	+			
		20	ETA	ND	ND	+			
		25	ETA	ND	ND	+			
2	F, 31	6	NP	ND	+[c]	ND	Pregnancy	OSEL, RIB	Alive
		20	NP	-	ND	-			
		21	ETA	-	ND	ND			
		24	NP	ND	+[c]	ND			
		24	ETA	-	-[b,f]	ND			
		26	ETA	ND	ND	-			
		40	NP	ND	+[c]	ND			
		56	NP	-	+[c]	ND			
		68	ETA	ND	ND	-			
		76	NP	-	ND	ND			
3	M, 55	7	NP	-	ND	ND	Asthma	OSEL	Expired
		9	NP	ND	+[c]	ND			
		11	ETA	-	ND	ND			
		12	BAL	-	+[c]	-			
4	F, 23	8	ETA	-	+[c]	ND	HTN, Pregnancy, Obesity	OSEL	Alive
		10	NP	ND	-[c]	ND			
		12	NP	-	ND	ND			
		24	NP	-	ND	ND			
5	F, 48	4	BAL	+	+[b]	+	COPD, bilateral lung transplant, CRI, Obesity	OSEL, ZAN	Expired
		10	BAL	+	+[b,e]	+			
6	F,	1	NP	ND	+[c]	ND	Asthma,	OSEL	Alive

	32	9	NP	-	-[c]	ND			
		11	NP	-	-[c]	ND			
7	F, 29	1	NP	-	+[c]	ND	Pregnancy	OSEL	Alive
		3	NP	-	ND	ND			
		8	NP	ND	-[c]	ND			
8	M, 48	2	NP	-	ND	ND	CHF	OSEL	Alive
		3	NP	ND	+[c]	ND			
		16	NP	-	ND	ND			
9	M, 23	5	NP	-	+[c]	-	Cystic Fibrosis	OSEL	Alive
		10	NP	-	ND	ND			
10	M, 62	7	NP	-	+[c]	ND	Multiple Myeloma, HTN	OSEL	Alive

NOTE.--Cx = culture; Tx = treatment; OSEL= oral oseltamivir; ZAN = inhaled zanamivir; RIB=oral ribavirin

NP=nasopharyngeal; ETA=endotracheal aspirate; BAL=bronchoalveolar lavage; CSF = cerebrospinal fluid; U = Unknown

ILD = interstitial lung disease; COPD = chronic obstructive pulmonary disease; CHF = congestive heart failure; HCT = hematopoietic cell transplantation; HTN = hypertension; CRI = Chronic Renal Insufficiency; DM = Diabetes Mellitus; ESRD = End Stage Renal Disease

ND=Not done; TFC = unsatisfactory, too few cells

[a] RT-PCR + = Probable or Detected for H1N1; [d] Viral culture + for influenza A isolates

[b] Santa Clara County Public Health Laboratory, San Jose, California

[e] California Department of Public Health, Richmond, California

[c] Focus Diagnostics, Cypress, California

[f] Unsatisfactory by California Department of Public Health, Richmond, CA

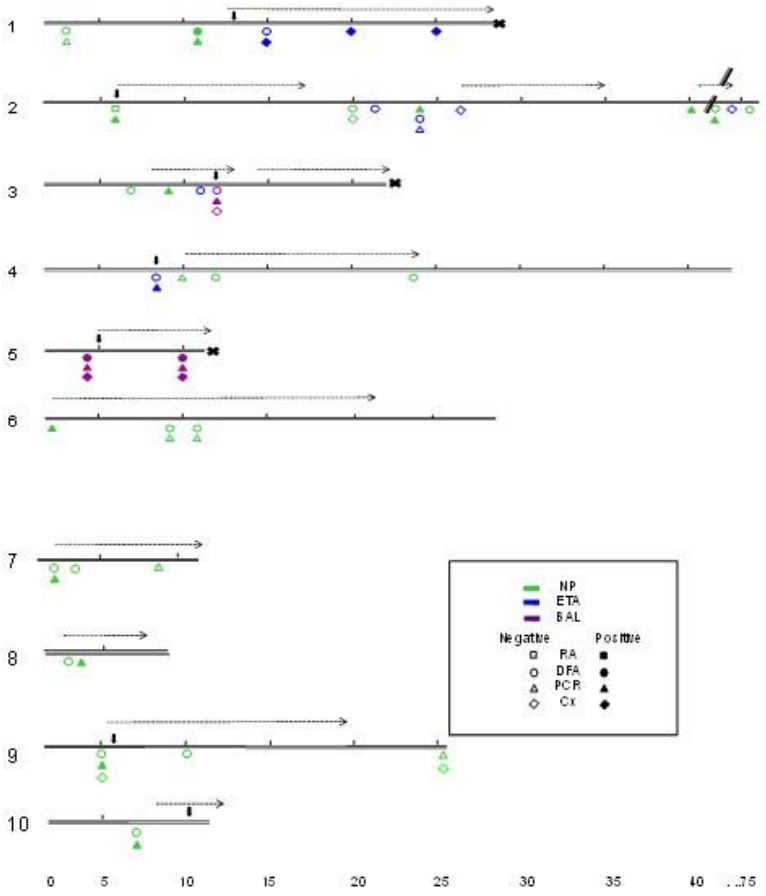

Figure 1. Clinical timelines of the 10 patients admitted to Stanford ICU with novel H1N1 infection. Time course is measured in days, with day 0 being onset of symptoms, as indicated at the bottom of the figure. Four patients (2, 4, 5, and 6) were admitted initially to outside hospitals before being transferred to Stanford ICU. Microbiological test results for viral testing are indicated by shapes including rapid influenza tests (squares), DFA tests (○ ●), RT-PCR (▲ ▲), and viral cultures (◇ ◆), and specimen site is indicated by color including nasopharynx (▬), endotracheal aspirates (▬), or bronchoalveolar lavage (▬). Solid shapes indicate positive tests; open shapes indicate negative tests. Arrows (⬇) indicate intubation. Days where patients received antiviral treatment are shown by the dashed arrows (--➔). Patients 1, 3, and 5 expired (✖), while the others survived and were discharged from the hospital. Patient 10 is still in the ICU as of the time of this writing. Patient 2's time course is interrupted after 40 days to allow her full hospital course to be included.