

So far, patient was afebrile after 4 weeks of voriconazole therapy. The post operative wound was cleaned with no pus discharged or slough.

**PP-068** In vitro antifungal susceptibility of *Candida* species isolated from patients with cancer

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**Background:** The genus *Candida* are one of the most frequent opportunistic pathogen in patients treated with immunosuppressive agents, cytotoxic and radiotherapy. Cytotoxic drugs have a major effect on the cell-mediated immunity and patients receiving such therapy are particularly vulnerable to opportunistic *Candida* infections. Epithelial cell of buccal mucosa are very sensitive to cancer therapies which may predispose the patients to candidiasis. Invasive candidiasis is frequently associated with primary buccal infections.

The virulence and antifungal susceptibility often vary among strains. In this study, we analyzed the in vitro antifungal susceptibility of 69 *Candida* species isolated from patients with cancer.

**Method:** The clinical strains were isolated from, lip, throat and tongue of patients with cancer in four university hospitals, Mazandaran Province. These strains were previously identified by phenotypic and molecular methods. In vitro antifungal susceptibility to amphotericin B, itraconazole, fluconazole, caspofungin was determined using the microdilution method described in the CLSI M27-A3 guideline.

**Result:** Fluconazole resistance was detected in 2 isolates (2.9%), among *Candida albicans* (2.5%) and *Candida glabrata* strains (8.3%). Itraconazole resistance was determined in 5 isolates (7.3%), among *Candida albicans* (5%) and *Candida glabrata* strains (20%). Amphotericin B resistance was detected in 3 isolates (4.3%), among *Candida albicans* (2.5%), *Candida glabrata* (8.3%). and *Candida tropicalis* (12.5%) strains. Caspofungin resistance was detected in one *Candida tropicalis* strain (12.5%).

**Conclusion:** In the present study, the antifungal activity of caspofungin was superior against clinical isolates of *Candida* species.

**PP-069** Neuraminidase N1 of the 2009 pandemic swine-origin influenza A virus H1N1 has a Group-2 specific active cavity

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The 2009 pandemic influenza seemingly spreads extremely quickly with worrisome mortalities and resembles some characteristics of the previous three pandemics (1918 Spanish-flu, 1957 Asian-flu and 1968 Hong Kong-flu). The virus was recognized as a new swine-origin H1N1 influenza A virus (S-OIV). Functional and structural characterization of the neuraminidase (NA) (09N1) might give us some clues about its pathogenesis and directs the drug application. Here the 09N1 was prepared in a baculovirus-based system and its enzymatic activity was verified *in vitro*. Its crystal structure

has been solved (1.9 Å) and the structure surprisingly shows a Group 2 active cavity, different from other known N1 structures which are all categorized into Group 1. The 09N1 structures in complex with substrate sialic acid, Oseltamivir (Tamiflu<sup>®</sup>) or Zanamivir (Relanza<sup>®</sup>) have also been solved at 1.8 Å, 1.7 Å and 1.9 Å respectively, showing typical binding modes and revealing the structural basis of the effectiveness of the NA-targeted drugs against the 2009 pandemic. This is the first solved NA structure derived from swine and the first complex structure with sialic acid for Group 1 members.

**PP-070** Application of extracorporeal membrane oxygenation in respiratory failure patients with H1N1 influenza: four cases report

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**Objective:** To evaluate the outcome of extracorporeal membrane oxygenation (ECMO) in critical ill patients with H1N1 influenza.

**Methods:** From NOV15 to Dec 25, 2009, four confirmed critical ill cases of pandemic H1N1 influenza were treated with ECMO.

**Results:** After using of ECMO, three patients evacuated the ECMO devices successfully, two (50%) patients died and two patients had been discharged from hospital. After treatment, the oxygenation index improved from 48–77mmHg (mean 59.8) to 122–254mmHg (mean 191.8). The complications included hemorrhage, catheter infection, and thrombosis in oxygenation membrane.

**Conclusion:** ECMO support may be helpful in some influenza cases with severe refractory pulmonary failure despite conventional management.

**PP-071** Clinical features of critically ill pregnant patients with influenza A (H1N1) infection

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**Objective:** To describe the clinical features of critical ill patients with H1N1 influenza in pregnant women.

**Methods:** From May 15 to Dec 20, 2009, seventeen cases of pandemic H1N1 in pregnant women were admitted to hospital and six were critical ill patients. The clinical features and supplemental data were analyzed.

**Results:** They ranged in age from 22–27 years (media 24.7), the gestational weeks on falling ill was 25–36 years (media 30.5), all were in the third trimester. Two patients died, other four patients had been discharged from hospital. The most common symptoms were fever, coughing, shortness of breath, and hemoptysis. Shortness of breath and hemoptysis were only found in critically ill patients. Anemia, hypoproteinemia, elevation of CRP, LDH, HBDH and decrease of T lymphocyte subpopulations count were easily found in critically ill patients. All patients developed pneumonia and subsequent acute respiratory distress syndrome, and four patients required mechanical ventilation, three patients required Extracorporeal Membrane Oxygenation (ECMO). Emergency caesarean delivery was performed in three patients for premature rupture of membranes (in two cases) and dead fetus in uterus (in one case) and one patient delivered a dead fetus herself in hospital. Other two patients continue their pregnancy after discharge from hospital.

**Conclusion:** Pregnant women might be at increased risk for critical ill complications from pandemic H1N1 virus infection, especially in the third trimester. The symptoms of shortness of breath and hemoptysis may be helpful in

detection of critically ill patients. Most critical care patients required mechanical ventilation and had a high case-fatality rate.

**PP-072** Comparative analysis of epidemiological and clinical characteristics with mild and severe influenza A (H1N1)

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**Objective:** To comparatively analyze the epidemiological and clinical characteristics of patients with severe influenza A (H1N1).

**Methods:** The clinical data were analyzed retrospectively from September 25, 2009 to November 23, 2009. Mild patients were 33 cases and severe patients were 32 cases.

**Results:** Patients in the 2 groups were mainly young people. The average age of mild group was 20.5±9.5 years old vs 26.5±20.0 years old in severe group. Occupational distribution of the 2 groups was mainly young staff and students. Fever, cough, expectoration and fatigue were prominent and characteristic features of the disease. The ratio of hyperpyrexia (≥39°C) and the duration of fever were statistically significant difference. There were no chest tightness, shortness of breath and vomiting in mild group. All patients in the 2 groups had throat congestion. No patients in mild group had complication, 24 cases in severe group had pneumonia and 8 cases had bronchial pneumonia. The ratio of abnormal of WBC count and absolute neutrophil count were statistically non-significant difference, but the abnormal of CK, LDH and liver function were statistically significant difference. All patients in severe group had abnormal chest X-ray examinations vs normal in mild group. All patients were successfully cured and discharged from hospital by oseltamivir phosphate capsules, Chinese patent medicine (Tanreqing injection) and symptomatic treatment. There were statistically significant difference of average hospital stay in the 2 groups.

**Conclusion:** Patients with influenza A (H1N1) are mainly young people. The complications of severe influenza A (H1N1) are pneumonia, heart damage and liver function abnormal and the process of the disease is in a extremely dangerous state. Though the ratio is declining, early detection, intensive care and active treatment should be all the same taken. Traditional Chinese medicine has comparative advantage and should be promoted.

**PP-073** An analysis of the clinical features of A/H1N1 influenza infection

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**Objective:** To reduce stuffs and patients being infected in hospitals, the clinical features of A/H1N1 influenza infection were studied.

**Methods:** The clinical data of 17 patients with A/H1N1 influenza were made a retrospectively investigation in June 2009, the data included the change of body temperature, blood routine and chest X-rays check, 18 patients with ordinary influenza were studied as the control group.

**Results:** There were not significant difference for the change of body temperature, blood routine (P>0.05). The chest X-rays check were normal in the two groups. All patients were confirmed positive for A/H1N1 RNA when they had fever, and were negative for A/H1N1 RNA when they had no fever in the A/H1N1 influenza group, those patients with ordinary influenza were all negative for A/H1N1 RNA when they had or not fever. These patients with A/H1N1

influenza or ordinary influenza had no complications, and all had good prognosis.

**Conclusion:** A/H1N1 influenza did not have any special clinical manifestations compared with ordinary influenza.

**PP-074** Small interfering RNA (siRNA) mediated inhibition of influenza A virus replication in mammalian cell line

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**Background:** Influenza A virus, since time immemorial, has posed an acute worldwide threat to human health and has been the cause of frequent epidemics and reoccurring pandemics. Various RNA interference (RNAi) studies have been carried out for the RNA-mediated RNA degradation in a sequence-specific manner. NS1 gene of influenza viruses plays a crucial role in inhibiting the interferon-mediated responses in the host.

**Methods:** We have studied the viral replication inhibition using siRNAs targeted against the conserved regions of the NS1 gene of influenza A Virus. The NS1 gene was cloned in pSecTag 2A vector and was co-transfected with 30, 40 and 50 pmoles of the designed siRNAs in MDCK cells. The same concentrations of siRNAs were also transfected with the whole virus (Influenza A/PR/8/34) to study the inhibition of replication. RT-PCR and Real-time RT-PCR assays followed by western blot analysis were performed to detect the inhibition of the expression of NS1 gene.

**Results:** All the tests confirmed an increase in inhibition of the expression of NS1 gene with an increase in the concentration of siRNA. The maximum inhibition (75%) of the virus replication was observed at 50 pmoles of siRNA.

**Conclusion:** Our study demonstrates that siRNA is able to cleave the target RNA at simulated physiological condition in a sequence specific manner. An increase in down-regulation of the cloned NS1 gene as well as a significant protection against influenza virus infection in MDCK cells was observed with an increase in siRNA concentration from 30–50 pm. The maximum inhibition of gene expression as well as viral replication inhibition was observed at 50pm concentration of siRNA.

**PP-075** Expression of neuraminidase protein of H1N1 swine-origin influenza A virus (S-OIV) in insect cells with a baculovirus expression system

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**Objective:** To construct the recombinant baculovirus expressing 2009 pandemic H1N1 swine-origin influenza A virus (S-OIV) Neuraminidase (NA) gene in insect cell.

**Methods:** The NA gene of Influenza A virus [A/California/VRDL98/2009 (H1N1)] was cloned into pGEM-T easy vector and then was ligated into baculovirus donor plasmid pFastBacHTa after cutting by *EcoRI* and *Hind III*. pFastBacHTa-NA was subsequently transformed into DH10Bac E. coli competent cells, which contained the baculovirus shuttle vector (Bacmid) and the helper plasmid to generate a recombinant bacmid. The recombinant baculovirus stock was prepared by transfecting the recombinant bacmid DNA into the Sf9 insect cell for protein expression after amplification. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting were performed to identify the antigenicity of the recombinant protein.