

SHORT REPORT

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Long-term respiratory follow-up of H1N1 infection

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Abstract

Background: The first case of 2009 pandemic influenza A (H1N1) virus infection was documented in our Hospital on 10th August 2009.

Methods and findings: Real-time reverse-transcriptase-polymerase-chain-reaction (RT-PCR) testing was used to confirm the diagnosis. All patients were treated with oseltamivir from the first day of hospitalization. Upon admission 12/44 had local patchy shadowing in their chest x-ray and additionally antibiotic regimen was added to these patients as pneumonia was suspected based on clinical evidence. In total 44 patients were hospitalized 15/44 had asthma, 6/44 COPD, 5/44 leukemia. Lung function was evaluated with forced vital capacity, forced expiratory volume in 1 sec and diffused carbon monoxide upon discharge and every 3 months, until 6 months of observation was completed after discharge. The purpose of this retrospective cohort study was to evaluate whether influenza A (H1N1) had an impact on the respiratory capacity of the infected patients.

Conclusions: An improvement of pulmonary function tests was observed between the first two measurements, implicating an inflammatory pathogenesis of influenza A (H1N1) to the respiratory tract. This inflammation was not associated with the severity or clinical outcome of the patients. All patients had a mild clinical course and their respiratory capacity was stable between the second and third measurement, suggesting that the duration of respiratory inflammation was two months. Early treatment with antiviral agents and vaccination represent the mainstay of management.

Introduction

The first human infections with the new influenza A (H1N1) virus were confirmed in April 2009 in America, but the infection had been rapidly spreading around the world and in June 2009 World Health Organization (WHO) declared a pandemic [1-5]. WHO had advised countries in the northern hemisphere to prepare for a second wave of pandemic spread [6]. To date, almost all countries in the world have confirmed a second wave of H1N1 virus [7,8]. For this reason a working group of WHO member states agreed on a pandemic influenza preparedness framework [9]. The reason for creating this framework began with the need for real time virological data exchange among laboratories in different

countries upon a pandemic outbreak [10,11]. The H1N1 virus has resulted from a triple recombination containing genes from human, avian, and swine influenza virus [12,13]. The incidence, clinical characteristics and factors affecting the patients outcome of the first wave have been well described in previous published studies [14-17]. However, while the numbers of positive H1N1 patients were lower, the hospitalisation rates were higher in comparison to the numbers and rates of 2009. In addition, the proportion of hospitalised cases admitted to intensive care units (ICU) was higher in 2010. The reason for these differences are not yet completely clear. There has been no obvious change in the severity of pandemic influenza A (H1N1) 2009 disease thresholds for hospital and ICU admission [8]. Three possible explanations were given. First, a new pandemic influenza A (H1N1) genetic variant predominated in the winter 2010 influenza season [7]. The Influenza A (H1N1)

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2009 strain had undergone mutation in the hemagglutinin. This mutation was associated with severe clinical outcome in comparison to the usual influenza A (H1N1) 2009 strain [7,18,19]. Secondly, resistance to oseltamivir developing from the first wave reached a peak in the 2010 pandemic [20,21]. Thirdly, it has been reported that this year's seasonal vaccine effectiveness was moderate, suggesting an insufficient protective effect against the second wave of influenza A (H1N1) [22-27].

In this retrospective study, we evaluated the respiratory capacity of 44 patients admitted to the Unit of Infectious Diseases (UID) of our hospital from 10th August 2009 to 15th November 2010. These were followed with Forced Vital Capacity (FVC), Forced Expiratory Volume in 1 sec (FEV1) and Carbon Monoxide Diffusing Capacity (DLCO) for a period of six months after their discharge. The rationale for the study was published data that H1N1 presents with hypoxemia and acute respiratory distress syndrome (ARDS) [14-17,28,29]. In addition, we examined the clinical characteristics of these patients.

Patients and methods

Methods of establishing H1N1 and precaution measures

Pharyngeal or nasopharyngeal swabs were taken upon admission in accordance with the protocol from the U.S. Centers for Disease Control and Prevention, as recommended by WHO and the average time between obtaining the samples and testing was 8-48 hours. The laboratory of the University Hospital of Alexandroupolis is a reference center for infectious diseases for more than 350.000 residents and one of the most important reference centers in Northern Greece. Confirmed case was defined by a positive result of a real-time reverse-transcriptase-polymerase-chain-reaction (RT-PCR) [30].

The pandemic (H1N1) 2009 influenza virus is thought to spread from person to person in the same way as seasonal influenza, where transmission occurs predominantly through droplets produced from coughing or sneezing. Indirect transmission may also occur through self-inoculation after contact with surfaces or objects contaminated with the virus from infected persons. The incubation period for pandemic (H1N1) 2009 influenza virus is understood to be approximately four days (range: 1-7 days). The period of communicability is estimated to be seven days in uncomplicated cases; however, it may be longer in children (up to 10 days) and other individuals in whom symptoms and virus shedding may persist (i.e. immuno-compromised and severely ill). Consistent with seasonal influenza, transmission of the pandemic (H1N1) 2009 influenza virus is most likely during the initial days of infection when cases are typically symptomatic and have higher viral loads [31].

The following isolation precautions were recommended for healthcare personnel who are in close contact with patients with suspected or confirmed 2009 H1N1 influenza. Close contact was defined as working within 6 feet of the patient or entering into a small enclosed airspace shared with the patient (e.g. average patient room). The standard precaution for all patient care was use of non-sterile gloves for any contact with potentially infectious material, followed by hand hygiene immediately after glove removal; use of gowns along with eye protection for any activity that might generate splashes of respiratory secretions or other infectious material.

Respiratory protection recommendation, according to CDC continues to be the use of respiratory protection that is at least as protective as a fit-tested disposable N95 respirator for healthcare personnel who are in close contact with patients with suspected or confirmed 2009 H1N1 influenza. This recommendation applies uniquely to the special circumstances of the H1N1 pandemic during the fall and winter of 2009-2010 and CDC will continue to revisit its guidance as new information becomes available, within this season if necessary.

The current recommendation is based on the unique conditions associated with the current pandemic, including low levels of population immunity to 2009 H1N1 influenza, availability of vaccination programs well after the start of the pandemic, susceptibility to infection of those in the age range of healthcare personnel, increased risk for complications of influenza in some healthcare personnel (e.g. pregnant women), and the potential for healthcare personnel to be exposed to H1N1 influenza patients because of their occupation [32].

Respiratory evaluation with FVC, FEV1 and DLCO was determined according to a joint statement based on the previous statements from the American Thoracic Society and European Respiratory Society [33]. Patients were evaluated the day of their discharge and every three months until six months all follow up were complete for each one. The concept was to evaluate whether these patients had clinical presentation of adverse effects of the virus on their respiratory capacity and to establish the duration of these effects.

Statistical analysis

Analysis was carried out with the use of SPSS statistical software package (SPSS version 17.01; SPSS, Chicago, IL, USA). Continuous variables were expressed as mean \pm SD or median (with interquartile ranges). For categorical variables, the percentages of patients in each category were calculated. Unpaired t-test was used in normally distributed parameters to compare the mean values between the two groups. A p value of less than 0.05 was considered to indicate statistical significance.

Results

Mean patient age was 36 years (14-65). The majority of the confirmed patients hospitalized were Caucasian male (28/44). All patients were treated immediately with oseltamivir (75-150 mg every 12 hours). Treatment with oseltamivir was continued for 4 to 10 days depending on the individual patient's clinical condition. In 12/44 patients, empiric antibiotic treatment (azithromycin + amoxicillin + clavulanic acid or ceftriaxone + quinolone) was given upon admission due to suspected secondary bacterial pneumonia elevated C-reactive protein, WBC count and local patchy shadowing on x-ray as reported in previous studies [34-36]. C-reactive protein and WBC count were elevated in these patients with local patchy shadowing on radiography (12/44 pts 27.2%).

Patients with radiological evidence of pneumonia were tested for serum procalcitonin (PCT) (36 patients in total) and *Legionella*/*Streptococcus pneumoniae* urinary antigens. Pneumonia score index (PSI) was applied and empirical antibiotic treatment was initiated according to the treating physician's clinical judgment. Patient's procalcitonin test was within normal range (0.05-0.1) in all patients. Additionally, urine antigen results for *Legionella* and *Streptococcus pneumoniae* and blood culture that were drawn were also negative. The pneumonia score index was evaluated in these patients and the range was between class II to IV for 12/44 patients [37].

Coexisting conditions were as follows: asthma 15/44 (34%), chronic obstruction disease 6/44 (13.6%), diabetes mellitus 8/44 (18.1%), coronary heart disease 10/44 (22.7%), lymphoma 5/44 (11.3%). Median laboratory values were: C-reactive protein 5.18 mg/dl, white blood count (WBC) 7.114, urea 26.18 mg/dl, creatinine 0.9 mg/dl, aspartate aminotransferase (AST) 27.09 IU/l, alanine aminotransferase (ALT) 24.48 IU/l. Abnormal liver function was observed in 7/44 (15.9%). Mean temperature and oxygen saturation upon admission were 39.06°C and 96.07%, respectively. Median PO₂ was 81.23 mmHg. Severe hypoxemia upon admission, defined as decreased partial pressure of oxygen in blood ≤ 60 mmHg with FiO₂ 21% [29], was observed in 7/44 patients. There was no thrombocytopenia observed. Median duration of hospitalisation and fever were 5.39 days and 2.45 days, respectively. Median body-mass index (BMI) was 30.23 kg/m². Obesity (BMI ≥ 30 kg/m² [38]) was noted in 11/44 and morbid obesity (BMI ≥ 40 kg/m²) was noted in 12/44 patients. Patient characteristics are presented in Table 1.

No further pharyngeal or nasopharyngeal swabs were taken during hospitalization or after treatment. The criteria for discharge were absence of hypoxemia and temperature ≤ 37°C for 1 day without antipyretic treatment [17].

Patient's respiratory capacity was evaluated with FVC, FEV1 and DLCO upon discharge and every three months until six months of observation was completed for each patient. The evaluation is demonstrated according to four categories which the authors selected based on the same underlying disease of the patients: a) chronic obstructive pulmonary disease, b) asthma, c) comorbidities (diabetes mellitus, coronary heart disease, and cancer), d) previously healthy patients. Whenever a patient had both an underlying respiratory disease and co-morbidity he was selected to be included in a group according to his major factor affecting his clinical outcome, preferably his underlying respiratory background. There was no association between radiographic data and pulmonary function tests. The mean values for FVC, FEV1 and DLCO are presented in Figures 1. A significant difference was observed between mean values of FEV1, FVC and DLCO between the evaluations upon discharge and the sixth month follow up (Table 2).

Discussion

We described a retrospective cohort study of 44 patients who were hospitalized with the pandemic 2009/10 Influenza A (H1N1) in the University Hospital of Alexandroupolis, Thrace from 10th August 2009 to 15th November 2010. So far, there has been no evaluation of H1N1 respiratory function. The correlation between the mechanisms and the impact that viruses have on the respiratory system has been established in previous published studies [14-17,28,29,39-44]. As might be anticipated, we found a major decreased respiratory capacity (FEV1 -10.205, FVC -9.909, DLCO -12.409) between the measurements taken upon discharge and in the six-month follow-up. This occurred due to the inflammatory effects of the virus through the respiratory tract. The differentiation of respiratory capacity also varied between the measurements taken upon discharge and the three-month follow-up, but it was observed that the variation was smaller in comparison to the sixth-month visit, which is possibly due to the time needed for the resolution of the inflammatory effects.

Patients with previously diagnosed respiratory disease such as COPD and asthma were well controlled on their treatment. H1N1 induced inflammation and was the factor that dysregulated their condition. WBC count and CRP were elevated in patients with suspected bacterial infection. Hypoxemia was more severe in patients with opacities on chest x-rays. The mean values in 7/44 patients with abnormal liver functions did not exceed 138 IU/L for ALT and 125 IU/L for AST. These patients did not present jaundice or other clinical signs of liver dysfunction. Patients with BMI ≥ 30 kg/m² were 20/44, implicating a mild clinical course overall, since

Table 1 Patients characteristics

Characteristic H1N1(+)	Upon admission			Upon Discharge		
	Mean	Range	(±SD)	Mean	Range	(±SD)
Age (years)	36	14-65	(14.7)			
Male/Female (n, %)	28/16 (63.6%/36.3%)					
Smokers						
(male/female) (n, %)	8/28 (28.6%) and 3/16 (18.8%)					
Non-Smokers						
(male/female)	20/28 (71.4%) and 13/16 (81.3%)					
BMI	30.23	20-45	(8.757)			
No.pts with obesity (male/female)	7/28 (25%) and 4/16 (25%)					
No.pts without obesity (male/female)	21/28 (75%) and 12/16 (75%)					
Co-existing conditions (n, %)						
Asthma	15/44 (34%)					
COPD	6/44 (13.6%)					
IPF	1/44 (2.2%)					
Lymphoma	5/44 (11.3%)					
Diabetes	8/44 (18.1%)					
Coronary Heart Disease	10/44 (22.7%)					
Outcomes-days						
Duration of fever in hospital under treatment	2.45	1-4	(0.875)			
Days of Hospitalization	5.39	3-12	(1.781)			
Days under oseltamivir regimen	5.25	4-10	(1.014)			
Adverse events (n, %)						
Abnormal liver function	7/44 (15.9%)					
Nausea, Diarrhea	4.5%					
Vomiting	1.4%					
Hypoxemia	1/44 (21.2%)					
CRP	5.18	0.26-17.38	(5.123)	1.33	0.16-4.53	(1.01)
WBC	7.114	3.340-10.950	(2172.160)	6.806	3340-10950	(2021.6)
Fever	39.06	37-40	(0.834)	37.8	36.3-39.2	(0.77)
CR	0.9	0.5-1.30	(0.17)	0.8	5-1	(0.12)
UR	26.18	11-41	(7.469)	25.11	12-40	(7.127)
SGPT	24.48	10-138	(26.37)	27.80	9-136	(26.719)
SGOT	27.09	14-125	(22.941)	24.14	9-57	(12.22)
Spo ₂ (FiO ₂ 21%)	96.07	89-99	(2.3)	96.98	94-99	(1.248)
PO ₂ (FiO ₂ 21%)	81.23	53-113	(14.586)	87.66	67-113	(12.473)
Abnormalities on chest radiograph (n, %)						
Local patchy shadowing	8/44 (18.1%)			6/44 (13.6%)		
Ground-glass opacities	3/44 (6.8%)			2/44 (4.5%)		
Interstitial abnormality	1/44 (2.2%)			1/44 (2.2%)		

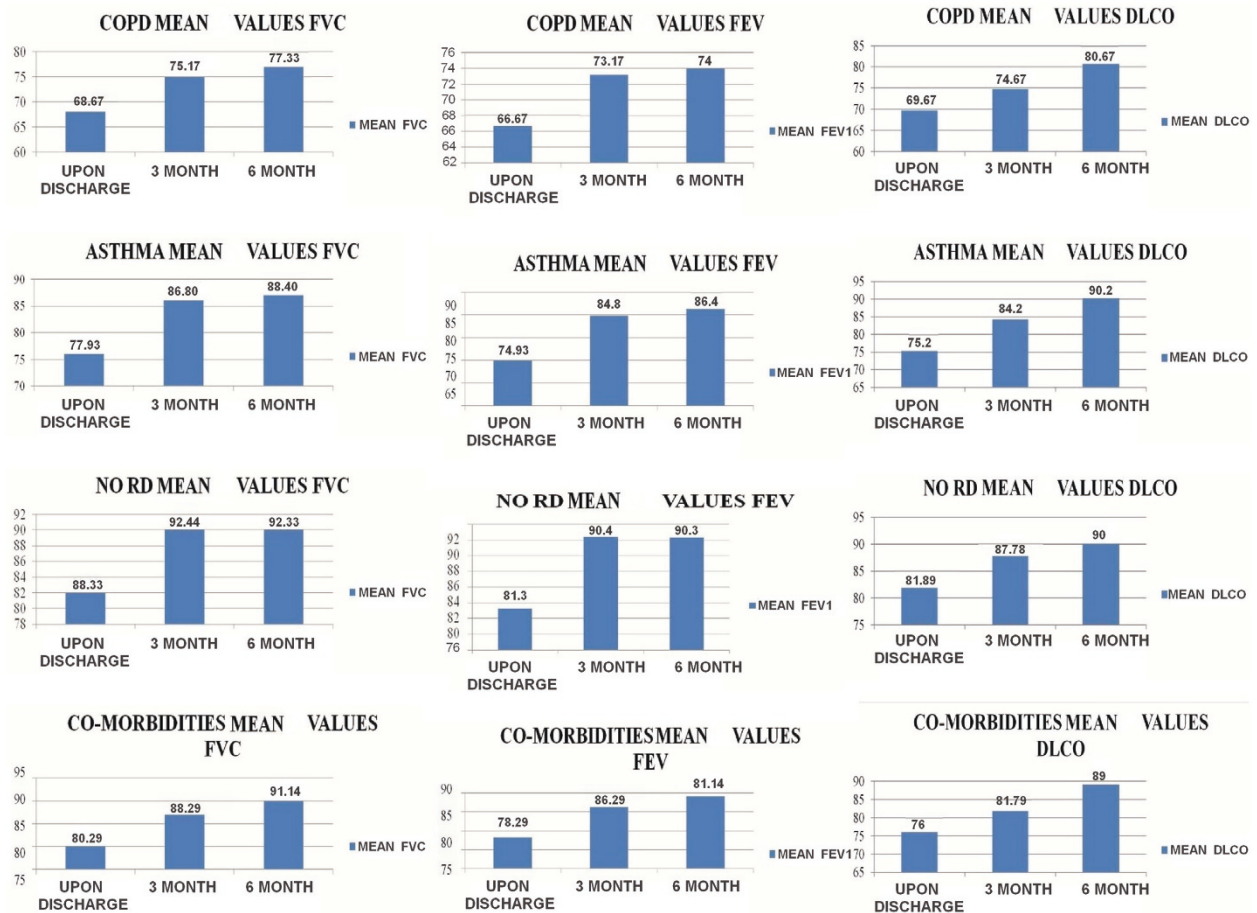


Figure 1 Forced Vital Capacity (FVC), Forced Expiratory Volume in 1 sec (FEV1), Carbon Monoxide Diffusing Capacity (DLCO) mean values for patients with Chronic Obstructive Pulmonary Disease (COPD), patients with Asthma, patients without any respiratory disease (NO RD), patients with co-morbidities.

BMI ≥ 30 is considered an adverse prognostic factor. The laboratory findings were not correlated with the respiratory functions, based on the fact that patients upon exacerbation or with opacities on chest x-ray would present a severe deterioration of their respiratory capacity. None of the patients had been previously vaccinated, but all patients received oseltamivir immediately on hospitalization and antibiotics upon suspicion of bacterial respiratory infection preventing an escalation of the inflammation and infection.

The mechanisms of H1N1-induced respiratory effects have received considerable attention [39-44]. Recent studies have shown that the high mortality rate of avian influenza virus infections is a consequence of an overactive inflammatory response and the severity of infection is closely related with virus-induced cytokine dysregulation. The most important feature of influenza A immune-pathogenesis is the appearance of “cytokine storm”, which is characterized by the extreme

production and secretion of numerous pro-inflammatory cytokines. This is responsible for the development of lethal clinical symptoms, such as massive pulmonary edema, acute bronchopneumonia, alveolar hemorrhage, reactive hemophagocytosis, and acute respiratory distress syndrome, associated with necrosis and tissue destruction. Numerous in vitro, in vivo and clinical studies have pointed out that A/H5N1 viruses are very strong inducers of various cytokines and chemokines (Tumor Necrosis Factor [TNF]-alpha, Interferon [IFN]-gamma, IFN-alpha/beta, Interleukin [IL]-6, IL-1, MIP [Macrophage Inflammatory Protein] -1, MIG [Monokine Induced by IFN-gamma], IP [Interferon-gamma-Inducible Protein]-10, MCP [Monocyte Chemoattractant Protein]-1, RANTES [Regulated on Activation Normal T-cell Expressed and Secreted], IL-8), in both humans and animals. The major cells implicated in the cytokine storm are macrophages and CD8+ T-lymphocytes, while the primary contributor cytokines are TNF-alpha, IL-6

Table 2 Statistical findings as mean values between follow ups.

DLCO	1DLCO	76.07	-6.750	.000
	2DLCO	82.82		
	1DLCO	76.07	-12.409	.000
	3DLCO	88.48		
	2DLCO	82.82	-5.659	.000
	3DLCO	88.48		
FEV1	1FEV1	76.18	-8.659	.000
	2FEV1	84.84		
	1FEV1	76.18	-10.205	.000
	3FEV1	86.39		
	2FEV1	84.84	-1.545	.001
	3FEV1	86.39		
FVC	1FVC	78.52	-8.318	.000
	2FVC	86.84		
	1FVC	78.52	-9.909	.000
	3FVC	88.43		
	2FVC	86.84	-1.591	.000
	3FVC	88.43		

and IFN-gamma. It has been detected that mutations of some viral genes (NS1, PB2, HA and NA) are responsible for the “cytokine storm”, by increasing the viral replication rate, expanding the tissue tropism, as well as facilitating the systemic invasion and the development of resistance against the host antiviral response. Glu92 and Ala149 mutations and carboxyl-terminal ESEV/EPEV motif of NS1 protein have been implicated as determinants of virulence for A/H5N1 strains. In addition, Lys627 mutation in PB2 protein, polybasic aminoacid mutations in the cleavage region of hemagglutinin (HA) polypeptide, and glycosylation and sialylation mutations in HA and neuraminidase (NA) proteins were found to enhance the immune-mediated pathology of highly virulent A strains [40-45]. Furthermore, in a preliminary analysis of macrophage gene expression data based on published studies, 64 genes in H1N1-infected cells showed at least 1.5-fold difference in expression level compared to mock infected cells in at least one time point [43]. Impressively, as many as 60 genes were thereby upregulated [43].

Given these important inflammatory processes, it is important to attempt patient prophylaxis. Several antiviral agents were tested both *in vitro* and/or *in vivo* and presented results implicating that the early use of such agents suppress the inflammation apart from being a therapy [43-49]. Antibiotics especially macrolides, which are well known for their anti-inflammatory and immunomodulatory properties were also tested. Clarithromycin inhibits the middle to late stage of the influenza virus replication cycle, resulting in inhibition of progeny virus production from the infected cells. Macrolides

could mediate this effect by inhibiting intracellular hemagglutinin HA0 proteolysis. The inhibitory effect on influenza virus replication of macrolides has been known since the 80s [50-54]. N-acetylcysteine (NAC) is a thiol-containing compound which non-enzymatically interacts and detoxifies reactive electrophiles and free radicals. NAC has already been shown to effectively protect human bronchial fibroblasts against the toxic effects of tobacco smoke condensates and the isolated perfused lung against the glutathione (GSH)-depleting effect of tobacco smoke. NAC has also been demonstrated to reduce the reactive oxygen intermediate hydrogen peroxide (H₂O₂) and to confer protection from the toxic effects of H₂O₂. *In vivo* studies, however, have demonstrated that orally administered NAC has very low bioavailability due to its rapid metabolism mainly to GSH but also other metabolites. Thus, even though NAC is very effective in protecting cells of different origins from the toxicity of reactive components in tobacco smoke and reactive oxygen species, a direct scavenging effect by NAC *in vivo*, does not seem likely. This holds especially true for oral administration. A more relevant mechanism *in vivo* for any protective effect of NAC may be attributable to its activity as a precursor of GSH, thereby facilitating its biosynthesis. GSH will then serve as the protective agent and detoxify reactive species both enzymatically and non-enzymatically [55,56]. Finally, the efficacy of vaccination for H1N1 has been documented by assessing antibody formation in the vaccinated elderly subjects from the 2009 pandemic in contrast to the younger ones who were not vaccinated. Nonetheless, antibodies from older subjects previous encounter with pandemic influenza A strains may be a confounding factor [57].

This study has a number of limitations. First, our patient series was small, but it should be borne in mind that since these H1N1 positive patients represent a population of 350.000 residents. Furthermore, diagnostic tests with procalcitonine, urine antigen and blood cultures were given only upon suspicion of an infection. The respiratory capacity tests represented a small number of patients in each group, and so no correlations could be performed between the four subgroups. In addition, the baseline condition of each patient was not evaluated; this was either because they did not have a respiratory underlying condition or because they were patients that admitted in our hospital for the first time.

Conclusions

Respiratory capacity differed between categories of patients at least for the first three months of observation, probably due to the inflammatory factors that are released with the influenza A (H1N1) infection and underlying disease. Importantly, the respiratory

inflammation lasted almost two months, as evidenced by the fact that respiratory capacity remained stable between the second and third measurement. Several treatment modalities can be administered to confer protection and suppress the inflammation. Vaccination and early treatment with antiviral agents represent the mainstay of management.

Acknowledgements

The authors would like to thank their colleagues in the Unit of Infectious Diseases in the University Hospital of Alexandroupolis, Greece.

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Authors' contributions

PZ, DS, NP, MM and TK wrote the manuscript and treated the patients, TCC performed statistical analysis, GK, IK, DM, and PS performed lung function tests, NC evaluated the chest x-rays and CT scan when necessary, KZ and EM provided useful insight. All authors read and approved the manuscript

Conflicts of interests

The authors declare that they have no competing interests.

Received: 6 June 2011 Accepted: 25 June 2011 Published: 25 June 2011

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doi:10.1186/1743-422X-8-319

Cite this article as: Zarogoulidis et al.: Long-term respiratory follow-up of H1N1 infection. *Virology Journal* 2011 **8**:319.

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