

# Risk Factors Associated with Severe Clinical Outcomes of Pandemic H1N1 Infection

## Abstract

The clinical outcome of pandemic H1N1 influenza infection is dependent on the interplay of virus and host factors. Influenza virus infections can range from relatively mild infections of the upper respiratory tract to fatal disease of the lower respiratory tract. A range of host factors play a role in the severity of clinical disease in pdm(H1N1)09 infection. With the exception of neonates age was directly proportional to the severity of clinical outcome; obesity, COPD and pregnancy play a key role in clinical outcome of infection. An important determinant in the pathogenesis of influenza infection is the tissue tropism of the influenza virus. The D222G and D222N mutations of pdm(H1N1)09 influenza appear to have an increased tropism for the tissues of the lower respiratory tract and are disproportionately associated with severe clinical disease. These  $\alpha$ -2-3 sialic acid tropic mutations, which have been associated with fatal cases of influenza infection, have also been preferentially selected for during sequential passage in embryonated eggs and viruses of avian origin.

## Keywords

Influenza; Pandemic; H1N1; pdm(H1N1)09; Oseltamivir

## Review Article

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## Abbreviations

ARDS: Acute Respiratory Distress Syndrome; BMI: Body Mass Index; CFR: Case Fatality Ratio; HA: Haemagglutinin; HPA: Health Protection Agency; ICU: Intensive Care Unit; NDA: No Detectable Antibody; pdm(H1N1)09: Pandemic 2009 H1N1 Influenza; SAE: Serious Adverse Event

## Introduction

The current H1N1 influenza vaccine strain A/California/7/2009 has a surprisingly high no detectable antibody (NDA) rate of approximately 52% in a population of 200 subjects screened (unpublished data). Studies have shown that the A/California/7/2009 strain of pandemic H1N1 influenza causes alveolar haemorrhage in the ferret model [1,2]; similar "swine flu" viruses A/Mexico/4482/2009 and A/Netherlands/602/2009 also presented severe pathology in the ferret model [3,4]. As a consequence it is clear that infection with this group of viruses represents a significant risk of a severe clinical outcome. Furthermore, the severe symptoms that are associated with a subset of A/California/7/2009 infections are atypical for previous seasonal influenza infections.

## Co-morbidities and their impact on clinical outcome of infection

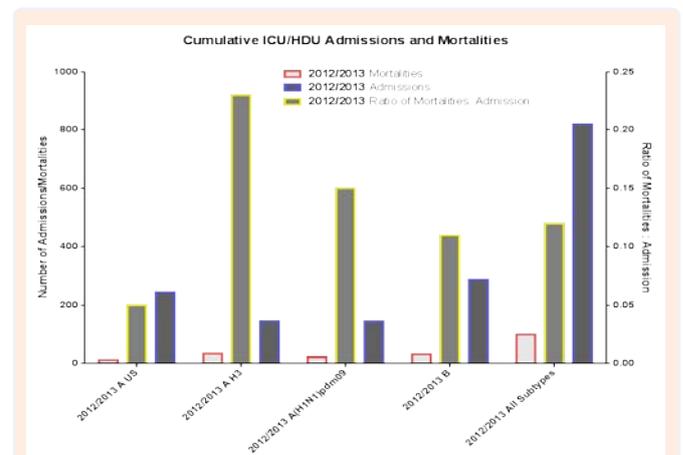
The clinical outcomes of infection by pdm(H1N1)09 are considerably affected by one or more host factors and co-morbidities. Here we discuss these co-morbidities and the impact that they have on clinical consequences to infection.

## Case fatality ratios

By July 2009 63,479 cases of influenza like illness were reported in Mexico; of these cases 11% (6,945) were confirmed as H1N1 influenza; 92% of confirmed positive cases (6,407)

were treated as outpatients, 7% (475) were admitted to hospital and survived and <1% died (63) [5]. During the early phase of the pandemic the case fatality ratio (CFR) was calculated to be approximately 0.4-0.6% [6]. In the UK an estimated 540,000 people had symptomatic H1N1 infection during a study period with a CFR of (0.026%) 26 deaths per 100,000 [7]. There is little published information for the CFRs of seasonal H1N1 or H3N2 influenza. However using data from the Health Protection Agency (HPA) we were able to determine the CFR for influenza infections admitted to intensive care units (ICU).

Figure 1 demonstrates that the CFR of H3N2 infections were higher than pdm(H1N1)09 infections over the period of 2012/2013.



**Figure 1:** Case ratios of hospital admissions and fatalities for pdm(H1N1)09, H3N2 and B virus infections over the season of 2012/2013.

## Symptoms

A meta-analysis of H1N1 pandemic influenza infection showed that the most common symptoms were cough (84.9%), fever (84.7%), headache (66.5%), runny nose (60.1%) and muscle pain (58.1%) [8].

## Factors associated with death or hospitalisation

Of those admitted to intensive care, up to 98% of patients and 91% of fatalities of pdm(H1N1)09 associated deaths were related to at least one co-morbidity [6,7,9-11] such as; age, chronic lung disease, obesity, pregnancy, diabetes, cardiac disease and immunosuppression. However a study of those admitted to ICUs in Australia and New Zealand found that 31.7% had no underlying condition [12].

**Age:** Generally patients with a non-severe clinical outcome were younger than those admitted to ICUs, who were also younger than those who died Table 1. According to one Canadian study, the median age was 23 years for all patients, 18 years for those with non-severe outcome, 34 years for those admitted to ICUs and those aged over 45 years were significantly more likely to die [13]. The greatest frequency of hospital admissions was in children aged 0-1 year followed by adults in the 50-64 year age group [12]. While studies suggest that children and young people had the highest cases of hospital admission, CFRs were highest in the over 65 year age group [5,7,9,10,13].

**Lung disease:** Chronic lung disease, asthma in particular, appears to be a common co-morbidity in severe cases of pdm(H1N1)09 infection including those requiring intensive care and those resulting in death [6,12,13]. Data on admissions to ICUs related to H1N1 influenza infection in Australia and New Zealand in 2009 showed that 32.7% of admissions had asthma or chronic pulmonary disease [12] and in Canada 41.1-47% of the critically ill also displayed these co-morbidities [11,13]. In Canada it was also shown that 22.6-38% of the critically ill were or had been smokers [11,14]. In California in 2009, 41% of fatal influenza cases had chronic lung disease [10] while in Germany it was reported to be 23.1% [15].

**Obesity:** Obesity has commonly been reported in ICU submissions and patients dying from pdm(H1N1)09 [6,11,12,15-18] and has been identified as a predictor of outcome in acute respiratory infection [19], possibly as a consequence of a defective cellular immune response to infection [20]. Of those admitted to an ICU in Australia and New Zealand in 2009, 28.6% (of which data was available) had a BMI of 35 kg/m<sup>2</sup> or greater [12]. In Canada 33.3% of the critically ill were obese (BMI ≥30) [11], and in one region of Canada the figure was as high as 62% [14]. In California, 66% of fatal adult cases involved obesity (BMI ≥30) [10] while in Germany the 23.1% of reported fatalities involved obesity (BMI >30) [15].

**Pregnancy:** Pregnancy has frequently been reported in cases of hospital admission related to pdm(H1N1)09 into ICUs. Pregnancy conveys a 4-5 fold increased rate of serious illness and hospitalisation with influenza [21]. During previous influenza outbreaks, pregnancy has also been associated with increased mortality and morbidity [22,23]; particularly if infection occurs during the third trimester [23]. Of those admitted to ICUs in Australia and New Zealand in 2009 9.1% were pregnant women [12] and 7.7-12% of the critically ill in Canada were pregnant [11,14]; in California 6% of fatal cases involved pregnancy [10] and in Germany there were 1.2% [15].

**Diabetes:** Diabetes triples the risk of hospital admission following pdm(H1N1)09 infection and quadruples the risk of ICU admission once hospitalised [24]. From the total number of admissions to ICUs in Australia and New Zealand in 2009, 16% had diabetes [12] as well as 20.8% of the critically ill in Canada [11] and with one region as high as 44% [14]. In Germany 17.2% of fatal cases were associated with diabetes [15].

**Heart disease:** Of the total number of admissions to ICUs in Australia and New Zealand in 2009, 10.5% had chronic heart failure [12] and 14.9% of the critically ill had cardiac disease in Canada [11] and in addition 23% of fatal cases in California [10] and 27.2% in Germany [15] had chronic heart disease.

**Respiratory failure and immunosuppression:** Although multi-organ failure has been reported in patients with

**Table 1:** Age specific indices of incidence of and mortality from pandemic pdm(H1N1)09 by Donaldson [7]. All population and case estimates rounded to nearest 1000.

Age Group (years)	Population *(1000s)	Cases (Estimated)		No of Deaths			Case Fatality Rate Deaths Per 100,000 Cases (Range)	Population Risk of Deaths. Deaths Per 100,000 Population (Exact 95% CI)
		No. Range (1000s)	Cumulative Incidence Per 100,000 Population	Total	Male	Female		
<1	641	7 (3-13)	1000	2	1	1	30 (2-260)	3.1 (0.3 to 11.3)
1- 4	2398	26 (12-53)	1100	7	3	4	27 (3-120)	2.9 (1.1 to 6.1)
5-14	5961	187 (86-381)	3100	20	7	13	11 (3-36)	3.4 (2.0 to 5.2)
15-24	6812	144 (67-297)	2100	17	8	9	12 (3-40)	2.5 (1.4 to 4.0)
25-44	14 460	125 (58-297)	850	37	21	16	30 (10-88)	2.6 (1.8 to 3.5)
45-64	12 661	45 (21-92)	350	29	21	8	65 (21-200)	2.3 (1.5 to 3.3)
≥ 65	8159	3 (1-5)	30	26	14	12	980 (300-3200)	3.2 (2.0 to 4.7)
All ages	51 092	536 (247-1097)	1100	138	75	63	26 (11-66)	2.7 (2.2 to 3.2)

\*From Office of National Statistics mid-population estimates 2007

pdm(H1N1)09 infections, the majority of severe illness relates to respiratory failure. Pneumonia can complicate clinical infection and is associated with severe clinical outcome. Immunosuppression was associated with the critically ill in 19.6-21% of the critically ill in Canada in 2009 [10,14] and 33% and 30% of fatal cases in California [14] and Germany [15].

### No known associated risk factor

During the 2009 pandemic there was a “substantial minority” of fatalities in patients that were previously healthy [7]. While the main cause of death was usually acute respiratory distress syndrome (ARDS) in patients with an underlying co-morbidity, there have been several reported cases of critical illness or death in patients, resulting from myocarditis, with no underlying condition [25-33].

There have also been cases of myocarditis resulting from seasonal influenza infection [34-36]. For patients with no underlying condition, the risk of a severe outcome was greatest among those 30-49 years old and those aged 60 and older [13]. The time from onset of illness to admission was 2-7 days with a median time of 3-4 days [12,13].

The classically reported influenza syndromes were:

- Viral pneumonitis or associated respiratory distress syndrome 48.8%
- Secondary bacterial pneumonia 20.3%
- Exacerbation of airflow limitation 13.9%
- Intercurrent illness or other illness 17.1% [13]

In general symptomology was similar to seasonal influenza, Webb et al. [12] did not find that the proportion of patients that died in hospital was any higher than that of patients who died from seasonal influenza who were admitted to an ICU [37]. With the exception of 0-1 year old group in general the severity of clinical outcome appeared to be directly proportional to age [5,7,9,10,12,13]. Other key risk factors leading to severe clinical outcomes were asthma, COPD and obesity [10-15] although there were a number of severe clinical cases with no known underlying condition [7,25-33].

### The virus

In addition to the host factors which play an important role in the clinical outcome of pdm(H1N1)09 infection, the virus genotype also plays a role in the severity of clinical outcome. Antiviral resistance can have an important role in the efficacy of treatment to infection while other mutations can alter the virus tropism leading to an increase in this verity of virus pathogenicity.

### Antiviral resistance

**H275Y:** Antiviral resistance to the neuraminidase inhibitor oseltamivir can be conferred by the well-characterised resistance mutation in the neuraminidase gene H275Y [38,39]. All current seasonal H1N1 (not H1N1 pdm) strains are genotypically (H275Y) and phenotypically resistant to oseltamivir [40,41].

A small proportion (1%-1.5%) of pdm(H1N1)09 virus strains are oseltamivir resistant, almost exclusively due to a H275Y mutation in the neuraminidase protein. It has been observed that the Y275 mutation was present as a minor variant in infected hosts before the onset of therapy. There is also evidence for the co-transmission of this drug-resistant variant with drug-susceptible viruses [42].

There is conflicting data on the presence of compensatory mutations. Four mutations have been identified that can either fully (R194G, E214D) or partially (L250P, F239Y) compensate for the fitness deficiency of the H274Y mutant. These compensatory mutations appear to restore replication efficiency of the virus which can be negatively impacted when conferring oseltamivir resistance. The compensatory effect of E214D is applicable in both seasonal influenza virus strain A/New Caledonia/20/1999 and 2009 pandemic swine influenza virus strain A/California/04/2009 [43].

**S247N:** S247N is a novel, naturally occurring N1 neuraminidase mutation that reduces oseltamivir sensitivity and greatly potentiates oseltamivir resistance in the context of the H275Y mutation. It has been shown that highly oseltamivir-resistant viruses pdm(H1N1)09 containing both the S247N and H275Y mutations transmit efficiently in the guinea pig transmission model [44] indicating the increased potential of proliferation of oseltamivir resistant strains. The prevalence of oseltamivir resistant viruses is increasing globally.

### Other resistance mutations

Originally, seasonal influenza H1N1 viruses were resistant to oseltamivir but mostly sensitive to amantadine. However, as a consequence of the emergence of the pdm(H1N1)09 virus that carried the S31N mutation in M2, virtually all currently circulating human influenza (pdm(H1N1)09 and H3N2 viruses are resistant to amantadine [45].

### Mutations affecting pathogenicity

**D222G\*:** The D222G mutation in the haemagglutinin gene has been shown to present an increased severity of infection and increased likelihood of a SAE [46-52]. The D222G mutation was reported by the WHO to have an overall prevalence of <1.8% but with a prevalence of 7.1% in fatal cases [53]. Kilander et al. [47] showed that the D222G mutation was isolated from 18% of (61) cases with severe influenza infection and from 0% of (205) cases with mild disease. Puzelli et al. [49] also showed that 5.8% of (52) severe cases and in 0.9% of (117) mild cases possessed the D222G mutation.

The mutation presents an alteration in the tissue tropism of the virus with a reduced affinity for  $\alpha$ 2-6 receptors and an increased affinity for  $\alpha$ 2-3 receptors [48,51,54-56] so increasing the affinity for the lower respiratory tract. This has been demonstrated by the higher frequency of D239G mutants detected in viruses isolated from patients with fatal outcomes and in isolates from lungs [57].

It has been shown in ferrets that viruses containing the D222G

mutation presented similar pathology compared to that of the wild type virus with respect to lethargy, weight loss, replication efficiency and transmission. In mice inoculated intranasally with D222G, a greater weight loss and viral replication were observed than with wildtype virus. In the human cell model the D222G virus replicated with reduced kinetics but to a higher titre [51].

Memoli et al. [58] isolated virus from the nasal wash of a severely ill immunocompromised patient at the time of diagnosis and from a bronchoalveolar lavage later in the course of infection. The nasal wash contained D222 and the lavage contained predominantly G222 virus. When compared to plaque purified G222 virus in a ferret model, the G222 predominant clinical isolate was the most pathogenic and developed most diversity during infection, indicating that increased diversity and not signal polymorphism was the most important in predicting pathogenic potential.

**D222E\*:** The mutation D222E in the haemagglutinin gene has been identified in a previously healthy 30 year old who suffered from Guillain-Barré Syndrome [59], however it is not clear whether the Guillain-Barré Syndrome was a result of the D222E. The D222E mutation was also detected by Puzelli et al. [49] & Miller et al. [60]. Puzelli et al. [49] found that the D222E mutation was evenly distributed between mild (31.6%) and severe cases (38.4%) of infection.

**D222N\*:** The mutation D222N in the hemagglutinin gene appears to be relatively rare but associated with severe disease; similarly to D222G, D222N appears to alter the tissue tropism of the virus for the lower respiratory tract [48,61]. Kilander et al. [47] found D222N mutations in only 4 of the 266 samples tested but it was present in 3 of those who had severe disease. Mak et al. [48] also found 4/458 samples analysed had the D222N mutation 3/4 cases had severe disease.

\*It should be noted that the D222G discussed by most publications is amino acid D239G when counted from the start codon of A/California/4(or 7)2009.

### Other mutations

The mutations S162R and T25P were also associated with severe influenza infection [62]. In addition, a meta-analysis of clinical, epidemiological and genomic data from the United States, Canada, United Kingdom, Australia and Japan based on official reports of public health agencies, found that 42.9% of individuals who died from laboratory-confirmed cases of the pdm(H1N1)09 were infected with a virus containing the Q310H mutation in its haemagglutinin [57].

### Environmental factors affecting genotype

It has been demonstrated that that serial passage of H1N1 virus in embryonated chicken eggs increased viral growth 32- to 64-fold, coincident with the increased prevalence of G222 or R223 in the HA protein [63]. Ramadhany et al. [64] also showed that the minor genotype of  $\alpha$ 2,3 tropic viruses in upper airways became dominant after passing through chicken eggs. The affinity for  $\alpha$ 2,3 has been exploited by making mutations

including D222G to produce a H1N1 live attenuated vaccine with enhanced replication in eggs [65].

The emergence of antiviral resistance to chemotherapy is always a cause for concern particularly when compensatory mutations can negate the biological fitness penalty for resistance [38,39,43]. The fact that viruses containing S247N and H275Y have been demonstrated to be transmissible in an animal model [44] indicates that there is scope for resistant mutations to proliferate in the field. This clearly shows the need for new therapies against these viruses and the careful control of the usage of current antiviral therapies.

While the D222G/N mutations are of low frequency they appeared to have a disproportionate impact on the pathogenesis of disease. These D222G/N do have the potential to impact on the clinical outcome of pdm(H1N1)09 infection even in patients with no underlying condition. D222E however did not appear to have any significant effect on the clinical progression of illness.

### Factors contributing to the pathogenic potential of pdm(H1N1)09

During the 2009 pandemic there was a minority of fatalities in patients with no known underlying risk factor [7]. As these fatal cases had no known risk factor, the genotype of the infecting virus may have affected the clinical outcome. It has been well established that mutations in the HA gene (D222G/N) can result in an increased severity of clinical outcome [46-52] and may explain the fatalities in these patients.

### Pre-existing immune response to pdm(H1N1)09 infection

It is likely that the severe clinical outcomes from the 2009 pandemic were of the result of a novel virus being exposed to a naive population. Recent papers indicate that cross reactive antibodies from non-pandemic H1N1 strains can elicit an immune response to pandemic influenza infection [66]. Jegaskanda et al. [67] showed that Macaques primed against seasonal H1N1 infection elicit a Natural Killer cell response producing IFN- $\gamma$  and CD107a in the presence of pandemic H1N1 HA. This response may lessen the severity of infection with pdm(H1N1)09 and could explain why the 2009 pandemic was not as severe as feared; with pre-existing cross reacting antibodies mitigating the more severe outcomes of infection.

### Studies to determine the pathogenic potential of D222G/N mutations

Currently there seems to be little or no data available on what proportion of a virus population needs to contain D222G/N mutations to present a severe clinical outcome. This could be determined by titrating a homogeneous stock of D222G/N produced by reverse genetics or plaque purification in a stock of D222 wild type virus. Viruses with differing proportions of D222G/N could then be analysed in the ferret challenge model to determine what levels of D222G/N were required to exert a severe pathogenic effect.

## Conclusion

Clearly patients with any underlying health conditions were at higher risk of a severe clinical outcome from infection with age playing an important role in the severity of the clinical outcome. Patients with no underlying condition, the risk of a severe outcome has been reported to be greatest among those aged 30-49 years old and those aged 60 and older [13].

## References

- Huang SSH, Banner D, Fang Y, Ng DCK, Kanagasabai T, et al. (2011) Comparative analyses of pandemic H1N1 and seasonal H1N1, H3N2, and influenza B infections depict distinct clinical pictures in ferrets. *PLoS ONE* 6(11): e27512.
- van den Brand JM, Stittelaar KJ, van Amerongen G, Rimmelzwaan GF, Simon J, et al. (2010) Severity of pneumonia due to new H1N1 influenza virus in ferrets is intermediate between that due to seasonal H1N1 virus and highly pathogenic avian influenza H5N1 virus. *J Infect Dis* 201(7): 993-999.
- Munster VJ, de Wit E, van den Brand JMA, Herfst S, Schrauwen EJA, et al. (2009) Pathogenesis and transmission of swine-origin 2009 A(H1N1) influenza virus in ferrets. *Science* 325(5939): 481-483.
- Pearce MB, Belser JA, Gustin KM, Pappas C, Houser KV, et al. (2012) Seasonal trivalent inactivated influenza vaccine protects against 1918 Spanish influenza virus infection in ferrets. *J Virol* 86(13): 7118-7125.
- Echevarría-Zuno S, Mejía-Arangurú JM, Mar-Obeso AJ, Grajales-Muñiz C, Robles-Pérez E, et al. (2009) Infection and death from influenza A H1N1 virus in Mexico: a retrospective analysis. *Lancet* 374(9707): 2072-2079.
- Domínguez-Cherit G, Lapinsky SE, Macias AE, Pinto R, Espinosa-Perez L, et al. (2009) Critically ill patients with 2009 influenza A(h1n1) in Mexico. *JAMA* 302(17): 1880-1887.
- Donaldson LJ, Rutter PD, Ellis BM, Greaves FE, Mytton OT, et al. (2009) Mortality from pandemic A/H1N1 2009 influenza in England: public health surveillance study. *BMJ* 339: b5213.
- Khandaker G, Dierig A, Rashid H, King C, Heron L, et al. (2011) Systematic review of clinical and epidemiological features of the pandemic influenza A (H1N1) 2009. *Influenza Other Respir Viruses* 5(3): 148-156.
- Nguyen-Van-Tam JS, Openshaw PJ, Hashim A, Gadd EM, Lim WS, et al. (2010) Risk factors for hospitalisation and poor outcome with pandemic A/H1N1 influenza: United Kingdom first wave (May-September 2009). *Thorax* 65(7): 645-651.
- Louie JK, Acosta M, Winter K, Jean C, Gavali S, et al. (2009) Factors associated with death or hospitalization due to pandemic 2009 influenza A(h1n1) infection in California. *JAMA* 302(17): 1896-1902.
- Kumar A, Zarychanski R, Pinto R, Cook DJ, Marshall J, et al. (2009) Critically ill patients with 2009 influenza A(h1n1) infection in Canada. *JAMA* 302(17): 1872-1879.
- ANZIC Influenza Investigators, Webb SA, Pettilä V, Seppelt I, Bellomo R, et al. (2009) Critical care services and 2009 H1N1 influenza in Australia and New Zealand. *N Engl J Med* 361(20): 1925-1934.
- Campbell A, Rodin R, Kropp R, Mao Y, Hong Z, et al. (2010) Risk of severe outcomes among patients admitted to hospital with pandemic (H1N1) influenza. *CMAJ* 182(4): 349-355.
- Zarychanski R, Stuart TL, Kumar A, Doucette S, Elliott L, et al. (2010) Correlates of severe disease in patients with 2009 pandemic influenza (H1N1) virus infection. *CMAJ* 182(3): 257-264.
- Wilking H, Buda S, von der Lippe E, Altmann D, Krause G, et al. (2010) Mortality of 2009 pandemic influenza A (H1N1) in Germany. *Euro Surveill* 15(49): 19741.
- Rello J, Rodríguez A, Ibañez P, Socias L, Cebrian J, et al. (2009) Intensive care adult patients with severe respiratory failure caused by Influenza A (H1N1)v in Spain. *Crit Care* 13(5): R148.
- Ugarte S, Arancibia F, Soto R (2010) Influenza A pandemics: Clinical and organizational aspects: The experience in Chile. *Crit Care Med* 38(Suppl 4): e133-e137.
- Lee EH, Wu C, Lee EU, Stoute A, Hanson H, et al. (2010) Fatalities associated with the 2009 H1N1 influenza A virus in New York city. *Clin Infect Dis* 50(11): 1498-1504.
- Hedlund J, Hansson LO, Ortqvist A (1995) Short- and long-term prognosis for middle-aged and elderly patients hospitalized with community-acquired pneumonia: impact of nutritional and inflammatory factors. *Scand J Infect Dis* 27(1): 32-37.
- Paich HA, Sheridan PA, Handy J, Karlsson EA, Schultz-Cherry S, et al. (2013) Overweight and obese adult humans have a defective cellular immune response to pandemic H1N1 influenza A virus. *Obesity (Silver Spring)* 21(11): 2377-2386.
- Carlson A, Thung SF, Norwitz ER (2009) H1N1 influenza in pregnancy: what all obstetric care providers ought to know. *Rev Obstet Gynecol* 2(3): 139-145.
- Dodds L, McNeil SA, Fell DB, Allen VM, Coombs A, et al. (2007) Impact of influenza exposure on rates of hospital admissions and physician visits because of respiratory illness among pregnant women. *CMAJ* 176(4): 463-468.
- Jamieson DJ, Honein MA, Rasmussen SA, Williams JL, Swerdlow DL, et al. (2009) H1N1 2009 influenza virus infection during pregnancy in the USA. *Lancet* 374(9688): 451-458.
- Allard R, Leclerc P, Tremblay C, Tannenbaum TN (2010) Diabetes and the severity of pandemic influenza A (H1N1) infection. *Diabetes Care* 33(7): 1491-1493.
- Cunha BA, Syed U, Mickail N (2010) Fulminant fatal swine influenza (H1N1): Myocarditis, myocardial infarction, or severe influenza pneumonia? *Heart Lung* 39(5): 453-458.
- Erden I, Erden EC, Ozhan H, Basar C (2011) Acute myocarditis mimicking acute myocardial infarction associated with pandemic 2009 (H1N1) influenza A virus. *Cardiol J* 18(5): 552-555.
- Gdynia G, Schnitzler P, Brunner E, Kandolf R, Bläker H, et al. (2011) Sudden death of an immunocompetent young adult caused by novel (swine origin) influenza A/H1N1-associated myocarditis. *Virchows Arch* 458(3): 371-376.
- Haessler S, Paez A, Rothberg M, Higgins T (2011) 2009 pandemic H1N1-associated myocarditis in a previously healthy adult. *Clin Microbiol Infect* 17(4): 572-574.
- Khambekar SK, Harden S, Corbett S (2011) Influenza A (H1N1) and myocarditis. *Heart* 97(19): 1630.
- Liao YC, Hsieh YC, Chang WC, Huang JL, Ting CT, et al. (2011) Fulminant myocarditis in an adult with 2009 pandemic influenza A (H1N1 influenza) infection. *J Chin Med Assoc* 74(3): 130-133.
- Mohite PN, Popov AF, Bartsch A, Zych B, Dhar D, et al. (2011) Successful

- treatment of novel H1N1 influenza related fulminant myocarditis with extracorporeal life support. *J Cardiothorac Surg* 6: 164.
32. Cabral M, Brito MJ, Conde M, Oliveira M, Ferreira GC (2012) Fulminant myocarditis associated with pandemic H1N1 influenza A virus. *Revista Portuguesa de Cardiologia* 31(7-8): 517-520.
  33. Davoudi AR, Maleki AR, Beykmohammadi AR, Tayebi A (2012) Fulminant myopericarditis in an immunocompetent adult due to pandemic 2009 (H1N1) influenza A virus infection. *Scand J Infect Dis* 44(6): 470-472.
  34. Engblom E, Ekfors TO, Meurman OH, Toivanen A, Nikoskelainen J (1983) Fatal influenza A myocarditis with isolation of virus from the myocardium. *Acta Medica Scand* 213(1): 75-78.
  35. Nolte KB, Alakija P, Oty G, Shaw MW, Subbarao K, et al. (2000) Influenza A virus infection complicated by fatal myocarditis. *Am J Forensic Med Pathol* 21(4): 375-379.
  36. Onitsuka H, Imamura T, Miyamoto N, Shibata Y, Kashiwagi T, et al. (2001) Clinical manifestations of influenza a myocarditis during the influenza epidemic of winter 1998-1999. *J Cardiol* 37(6): 315-323.
  37. Li G, Yilmaz M, Kojicic M, Fernández-Pérez E, Wahab R, et al. (2009) Outcome of critically ill patients with influenza virus infection. *J Clin Virol* 46(3): 275-278.
  38. Collins PJ, Haire LF, Lin YP, Liu J, Russell RJ, et al. (2009) Structural basis for oseltamivir resistance of influenza viruses. *Vaccine* 27(45): 6317-6323.
  39. Brookes DW, Miah S, Lackenby A, Hartgroves L, Barclay WS (2011) Pandemic H1N1 2009 influenza virus with the H275Y oseltamivir resistance neuraminidase mutation shows a small compromise in enzyme activity and viral fitness. *J Antimicrob Chemother* 66(3): 466-470.
  40. Meijer A, Lackenby A, Hungnes O, Lina B, van-der-Werf S, et al. (2009) Oseltamivir-resistant influenza A (H1N1) virus, Europe, 2007-08 season. *Emerg Infect Dis* 15(4): 552-560.
  41. Whitley RJ, Boucher CA, Lina B, Nguyen-Van-Tam JS, Osterhaus A, et al. (2013) Global assessment of resistance to neuraminidase inhibitors, 2008-2011: The Influenza Resistance Information Study (IRIS). *Clin Infect Dis* 56(9): 1197-1205.
  42. Ghedin E, Holmes EC, DePasse JV, Pinilla LT, Fitch A, et al. (2012) Presence of oseltamivir-resistant pandemic a/h1n1 minor variants before drug therapy with subsequent selection and transmission. *J Infect Dis* 206(10): 1504-1511.
  43. Wu NC, Young AP, Dandekar S, Wijersuriya H, Al-Mawsawi LQ, et al. (2013) Systematic identification of H274Y compensatory mutations in influenza A virus neuraminidase by high-throughput screening. *J Virol* 87(2): 1193-1199.
  44. Seibert CW, Rahmat S, Krammer F, Palese P, Bouvier NM (2012) Efficient transmission of pandemic H1N1 influenza viruses with high-level oseltamivir resistance. *J Virol* 86(9): 5386-5389.
  45. Dapat IC, Dapat C, Baranovich T, Suzuki Y, Kondo H, et al. (2012) Genetic characterization of human influenza viruses in the pandemic (2009-2010) and post-pandemic (2010-2011) periods in Japan. *PLoS ONE* 7(6): p. e36455.
  46. Antón A, Marcos MA, Martínez MJ, Ramón S, Martínez A, et al. (2010) D225G mutation in the hemagglutinin protein found in 3 severe cases of 2009 pandemic influenza A (H1N1) in Spain. *Diagn Microbiol Infect Dis* 67(2): 207-208.
  47. Kilander A, Rykkvin R, Dudman SG, Hungnes O (2010) Observed association between the HA1 mutation D222G in the 2009 pandemic influenza A(H1N1) virus and severe clinical outcome, Norway 2009-2010. *Euro Surveill* 15(9): 19498.
  48. Mak GC, Au KW, Tai LS, Chuang KC, Cheng KC, et al. (2010) Association of D222G substitution in haemagglutinin of 2009 pandemic influenza A (H1N1) with severe disease. *Euro Surveill* 15(14): 19534.
  49. Puzelli S, Facchini M, Spagnolo D, De Marco MA, Calzoletti L, et al. (2010) Transmission of hemagglutinin D222G mutant strain of pandemic (H1N1) 2009 virus. *Emerg Infect Dis* 16(5): 863-865.
  50. Baldanti F, Campanini G, Piralla A, Rovida F, Braschi A, et al. (2011) Severe outcome of influenza A/H1N1/09v infection associated with 222G/N polymorphisms in the haemagglutinin: a multicentre study. *Clin Microbiol Infect* 17(8): 1166-1169.
  51. Belser JA, Jayaraman A, Raman R, Pappas C, Zeng H, et al. (2011) Effect of D222G mutation in the hemagglutinin protein on receptor binding, pathogenesis and transmissibility of the 2009 pandemic H1N1 influenza virus. *PLoS ONE* 6(9): e25091.
  52. Houg HS, Garner J, Zhou Y, Lyons A, Kuschner R, et al. (2012) Emergent 2009 influenza A(H1N1) viruses containing HA D222N mutation associated with severe clinical outcomes in the Americas. *J Clin Virol* 53(1): 12-15.
  53. WHO (2010) Preliminary review of D222G amino acid substitution in the haemagglutinin of pandemic influenza A (H1N1) 2009 viruses. *Weekly epidemiological record* 85(4): 21-28.
  54. Chutinimitkul S, Herfst S, Steel J, Lowen AC, Ye J, et al. (2010) Virulence-associated substitution D222G in the hemagglutinin of 2009 pandemic influenza A(H1N1) virus affects receptor binding. *J Virol* 84(22): 11802-11813.
  55. Abed Y, Pizzorno A, Hamelin ME, Leung A, Joubert P, et al. (2011) The 2009 pandemic H1N1 D222G hemagglutinin mutation alters receptor specificity and increases virulence in mice but not in ferrets. *J Infect Dis* 204(7): 1008-1016.
  56. Chan PKS, Leeb N, Joyntd GM, Choie KW, Cheunga JLK, et al. (2011) Clinical and virological course of infection with haemagglutinin D222G mutant strain of 2009 pandemic influenza A (H1N1) virus. *J Clin Virol* 50(4): 320-324.
  57. Ginsky GV (2010) Genomic analysis of pandemic (H1N1) 2009 reveals association of increasing disease severity with emergence of novel hemagglutinin mutations. *Cell Cycle* 9(5): 958-970.
  58. Memoli MJ, Bristol T, Proudfoot KE, Davis AS, Dunham EJ, et al. (2012) *In vivo* evaluation of pathogenicity and transmissibility of influenza A(H1N1)pdm09 hemagglutinin receptor binding domain 222 intrahost variants isolated from a single immunocompromised patient. *Virology* 428(1): 21-29.
  59. Cortese A, Baldanti F, Tavazzi E, Carnevale L, Minoli L, et al. (2012) Guillain-Barré syndrome associated with the D222E variant of the 2009 pandemic influenza A (H1N1) virus: case report and review of the literature. *J Neurol Sci* 312(1-2): 173-176.
  60. Miller RR, MacLean AR, Gunson RN, Carman WF (2010) Occurrence of haemagglutinin mutation D222G in pandemic influenza A(H1N1) infected patients in the West of Scotland, United Kingdom, 2009-10. *Euro Surveill* 15(16): 19546.
  61. Houg HS, Garner J, Zhou Y, Lyons A, Kuschner R, et al. (2012) Emergent 2009 influenza A(H1N1) viruses containing HA D222N mutation associated with severe clinical outcomes in the Americas. *J Clin Virol* 53(1): 12-15.

62. Melidou A, Gioula G, Exindari M, Chatzidimitriou D, Diza E, et al. (2010) Molecular and phylogenetic analysis of the haemagglutinin gene of pandemic influenza H1N1 2009 viruses associated with severe and fatal infections. *Virus Res* 151(2): 192-199.
63. Yasugi M, Nakamura S, Daidoji T, Kawashita N, Ramadhany R, et al. (2012) Frequency of D222G and Q223R hemagglutinin mutants of pandemic (H1N1) 2009 influenza virus in Japan between 2009 and 2010. *PLoS ONE* 7(2): e30946.
64. Ramadhany R, Yasugi M, Nakamura S, Daidoji T, Watanabe Y, et al. (2012) Tropism of pandemic 2009 H1N1 influenza A virus. *Front Microbiol* 3: 128.
65. Chen Z, Wang W, Zhou H, Suguitan AL Jr, Shambaugh C, et al. (2010) Generation of live attenuated novel influenza virus A/California/7/09 (H1N1) vaccines with high yield in embryonated chicken eggs. *J Virol* 84(1): 44-51.
66. Ellebedy AH, Ducatez MF, Duan S, Stigger-Rosser E, Rubrum AM, et al. (2011) Impact of prior seasonal influenza vaccination and infection on pandemic A (H1N1) influenza virus replication in ferrets. *Vaccine* 29(17): 3335-3339.
67. Jegaskanda S, Weinfurter JT, Friedrich TC, Kent SJ (2013) Antibody-dependent cellular cytotoxicity is associated with control of pandemic H1N1 influenza virus infection of macaques. *J Virol* 87(10): 5512-5522.