

# Epidemiologic analysis of virulent mutations of A/H1N1pdm09 in hospitalized and out-patients during 2013-14 & 2014-15 epidemic seasons in Spain

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## Introduction & Objective

Flu A viruses produce a variable percentage of severe/hospitalized cases that oscillates between 2-10 cases/100,000 inhabitants in Spain depending of each influenza epidemic. It is necessary to determine the genetic characteristics of flu viruses, focusing on designing diagnostic tools able to predict the clinical evolution of each patient and for specific management of medical treatments and care. The aim of this study is to describe the differences of genetics characteristics of flu A/H1N1pdm09 virus related to the severity of patients.

## Material/methods

A prospective multicentric study was performed recruiting 64 respiratory samples from hospitalized patients diagnosed of A/H1N1pdm09 influenza virus respiratory infection in several hospitals from Valladolid, Barcelona and Madrid (Spain) during 2013-14 and 2014-15 influenza epidemics. It was also recruited 11 out-patients with positive diagnostic for A/H1N1pdm09. The 8 genes of influenza virus were amplified with *PathAmp™ FluA* reagents and deep sequenced using an *Ion Torrent PGM* platform (Figure 1) (*Life Technologies*). The sequences obtained were aligned by using *Bioedit 7.2.3* software, and mutations were analyzed according to the clinical features of patients with *FluSurver* free software.

## Results

Mutation E391K conferring higher virulence was observed widely distributed in both type of patients in HA gene, while other mutations like V6A, L8M, Q297K and L543M, without known effect, were only detected in hospitalized. Mutation D199N in NA gene conferring mild Oseltamivir resistance was found in two hospitalized patients, one of them recovering in a week with Tamiflu 2X75mg/day while the other required ICU assistance and flu viral load was detected after one month with the same antiviral treatment. Adamantane resistance in M gene was observed widely distributed in hospitalized and out-patients as 31N, V27I and V27A mutations. In PB2 gene, T676N mutation conferring higher viral virulence was observed in two hospitalized patients, one of them with a persistent viral shedding. N205S mutation, related with higher virulence in ferret models, was observed in NS1 gene in all patients. Regarding this gene, it was also documented an ICU patient who died with flu virus carrying G201E and E217D mutations in NS1, resulting in a higher virulence of the virus. It was not found significant results in NP, PA or PB1 genes.



Figure 1. Ion Torrent PGM platform used for flu sequencing

## Conclusions

It was not observed a widely distributed type of mutations that could induce a worse clinical progression of the hospitalized respect to the out-patients. It is estimated, therefore, that the immune system of each individual could be the most important predisposing factor for poor outcome than the genetic of the virus itself. Moreover, it must take into account the emergence of Tamiflu resistance mutations different than H275Y, as D199N mutation, present in some samples from these study.