Serological study of the 2009 pandemic due to influenza A H1N1 in the metropolitan French population

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Abstract

We looked for evidence of antibodies to the 2009 influenza A/H1N1 pandemic virus in panels of sera from individuals living in metropolitan France, obtained either before, during or after the epidemic, using standard haemagglutination inhibition and microneutralization tests. The difference between seroprevalence values measured in post- and pre-epidemic panels was used as an estimate of seroconversion rate in different age groups (23.4% (0–24 years, age-group 0); 16.5% (25–34); 7.9% (35–44); 7.2% (45–54); 1.6% (55–64); and 3.1% (>65)), confirming that the distribution of cases in different age groups was similar to that of the seasonal H1N1 virus. During the pre-pandemic period low-titre cross-reactive antibodies were present in a large proportion of the population (presumably acquired against seasonal H1N1) whereas cross-reactive antibodies were detected in individuals over the age of 65 years with significantly higher prevalence and serological titres (presumably acquired previously against Spanish flu-related H1N1 strains). Clinical data and analysis of post-pandemic seroprevalence showed that few of these latter patients were infected by the influenza virus during the epidemic. In contrast, the majority of both clinical cases and seroconversions were recorded in the 0–24 age group and a global inverse relationship between prevalence of antibodies to pH1N1 in the pre-pandemic period and rate of seroconversion was observed amongst age groups. Our results emphasize the complex relationships involved in antigenic reactivity to pandemic and seasonal H1N1 viral antigens; hence the difficulty in distinguishing between low-titre specific and cross-reactive antibodies, establishing precise seroprevalence numbers and fully understanding the relationship between previous immunity to seasonal viruses and protection against the novel variant.

Keywords: Epidemiology, H1N1, haemagglutination, microneutralization, pandemic, seroconversion
Original Submission: 9 September 2010; **Revised Submission:** 1 April 2011; **Accepted:** 3 April 2011
Editor: T. A. Zupanc
Article published online: 25 April 2011
Clin Microbiol Infect 2012; **18:** 177–183
10.1111/j.1469-0691.2011.03545.x

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Introduction

A novel influenza virus, A/H1N1, emerged in Mexico in April 2009 (pH1N1(2009), referred to here as pH1N1). In June, the World Health Organization declared a pandemic alert due to its rapid global dispersal. Significant differences between the antigenic structure of this novel variant and that of H1N1 seasonal viruses (referred to here as sH1N1) that circulated during the previous decades, were identified [1–3]. This agreed with the observed epidemiological dissemination of the disease that first hit western countries during the summer of 2009 and with the first serological studies that appeared to show limited cross-reactivity between pandemic and seasonal H1N1 viruses [1,4–10], predicting limited protection following vaccination based on seasonal influenza vaccine [5,9].

To date, few seroepidemiological studies of the first and second waves of the 2009 pandemic have been performed [11–16], but the accumulating data imply that the antigenic relationship between the pandemic virus and previously circulating H1N1 viruses is more complex than was initially realised: antigenic relationships between the haemagglutinin of the pandemic virus and that of the 1918 H1N1 virus were

confirmed by structural studies [3,17–21], but a significant correlation was observed between neutralization of pandemic A/H1N1 and neutralization of a standard seasonal A/H1N1 strain, and significantly higher pH1N1 neutralizing titres were detected in subjects who had previously received vaccination against seasonal influenza in 2008–2009 [22].

Here, we have examined the antibody repertoire of the pandemic A/H1N1 virus in individuals, using panels of pre-, per- and post-epidemic sera from French metropolitan populations. In parallel, we tested pre-pandemic sera for the presence of antibodies to the recently circulating H1N1 seasonal virus.

We present the first dataset, which allows us to propose an estimate of the seroconversion rate in the French metropolitan population during the 2009 pandemic waves and discuss the distribution of cases in age groups in the light of the complex antigenic cross-relationships between pandemic and seasonal H1N1 viral antigens.

Materials and Methods

Ethical issues

This study was approved by the Departmental (IFR48) Ethics Committee for archival hospital panels and by the 'Comité de Protection des Personnes Ile de France' for per-pandemic samples (including patient consent). All information in databases was made anonymous.

Populations studied

Pre-epidemic serum samples. One thousand six hundred and ninety-three sera collected in 2007–2008 (archival material randomly selected from the serum library of the Public Hospitals of Marseilles) were tested for antibody to pH1N1, including a subpopulation of 1020 samples also tested for sH1N1. The distribution in age groups is indicated in Table 1.

Post-epidemic samples. Similarly, 1396 archival sera sampled after the end of the pandemic wave in France (weeks 51-2009 to 12-2010) were tested for antibody to pH1N1.

Per-epidemic samples. Sera were collected between weeks 45-2009 and 12-2010 from 1541 women (not vaccinated against pH1N1) tested during the first term of pregnancy in French private biology laboratories (RBML network) for toxoplasmosis (95% in the 20–39 years age group; median age, 30). The geographical origin of samples is shown in Fig. 1. Sera were tested for antibody to pH1N1.

Haemagglutination inhibition (HI) assays

Viral antigen. This was prepared from a phosphate buffer saline dilution of influenza virus cell culture supernatant medium, conserved either at −80°C (HI-assay) or freeze-dried in the presence of sucrose 0.2 M (microneutralization assay). Strains used were: (i) pH1N1: OPYFLU-1, isolated in Marseille in early May 2009 [8,23], and (ii) sH1N1: MRS-2007 (closely related to strain A/Paris/6/2007(H1N1)).

HI-assay. This was conducted in a Bio-Safety Level 3 laboratory using 5.33 haemagglutinating units of non-inactivated antigen, serial dilutions (1/10–1/1280) of heat-inactivated sera, group O human erythrocytes (French Blood Bank), and Eppendorf epMotion working stations. The HI-titre was determined as the highest dilution providing clear inhibition of haemagglutination. All experiments included the same neg-

TABLE 1. Seroprevalence of pandemic H1N1 in pre- and post-epidemic panels, using the haemagglutination inhibition (HI) method

	Pre-epidemic samples (2007–2008)		Post-epidemic samples (2010)		Difference (CI)		Cases in FMP	
Total	*N* = 1693		*N′* = 1396			FMP* 62.8	*N″**(CI)	Distribution 100%
Age		%HI ≥1/40 (CI)		%HI ≥1/40 (CI)	Δ ≥1/40 (CI)		$\sum$ = 7.5	
0–24	383	40.47 (35.55–45.39)	318	63.84 (58.56–69.12)	23.37 (18.09–28.65)	19.4	4.5 (3.5–5.6)	59.8%
25–34	299	43.48 (34.99–51.96)	235	60.00 (50.54–69.46)	16.52 (7.06–25.98)	7.8	1.3 (0.5–2.0)	17.0%
35–44	267	45.32 (33.25–57.38)	231	53.25 (40.25–66.25)	7.93 (−5.07–20.93)	8.9	0.7 (0.0–1.9)	9.3%
45–54	190	51.58 (33.60–69.56)	194	58.76 (41.23–76.29)	7.18 (−10.8–25.16)	8.5	0.6 (0.0–2.1)	8.1%
55–64	205	58.54 (38.03–79.04)	208	60.10 (39.86–80.33)	1.56 (−18.95–22.07)	7.7	0.1 (0.0–1.7)	1.6%
65–100	349	68.77 (51.50–86.03)	210	71.90 (50.32–93.49)	3.14 (−18.45–24.73)	10.5	0.3 (0.0–2.6)	4.3%

The difference between the 2007–2008 baseline and the 2010 seroprevalence was used as an estimate of the seroconversion rate (Δ ≥1/40).
FMP, French metropolitan population for year 2009 (source INSEE); *N*, number of samples tested from pre-pandemic panel; *N′*, number of samples tested from post-pandemic panel; *N″*, estimates of the number of cases in the French metropolitan population; %HI ≥1/40, proportion of samples with a pH1N1 HI-titre ≥1/40; Δ ≥1/40, difference of seroprevalence (titre ≥1/40) between pre- and post-pandemic panels. CI, 95% confidence interval calculated according to the Wald method without continuity correction.
*Expressed in millions.
Distribution: estimate of the distribution of cases in the different age classes of the FMP, based on N″ values.