Immunogenicity of the Pandemrix A(H1N1)2009 influenza vaccine in hemodialysed and renal transplanted patients

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A. Hombrouck[1], N. Broeders[2], L. Labriola[3], D. Abramowicz[2], M. Jadoul[3], P. Goubau[3], S. Van Gucht[1], B. Brochier[1] and I. Thomas[1]

[1] Scientific Institute of Public Health, Brussels, Belgium
[2]Erasmus hospital, Brussels, 1070, Belgium
[3]University hospital St. Luc, Brussels, 1200, Belgium

Introduction

Swine-origin pandemic human influenza A(H1N1)2009 rapidly spread around the world since its initial reporting on the 25th of April 2009. To counter the virus, different vaccines were developed. In Belgium, the Pandemrix vaccine of GSK was used. Part of the Belgian population was vaccinated, especially those belonging to risk groups (immunodepressed patients, healthcare providers, patients with chronic disease, pregnant women, ….). In general, vaccine immunogenicity is less effective in immunodepressed patients than in immunocompetent patients. In this study the immunogenicity of the Pandemrix vaccine was investigated by measuring neutralizing antibodies against A(H1N1)2009 in sera collected from immunocompetent and immunodepressed patients such as hemodialysed and renal transplanted patients.

Patients and methods

Patients In total 106 hemodialysed patients and 112 renal transplanted patients were recruited. Thirty-two volunteers without health disorder resulting in immunodeficiency were recruited as controls: 10 close relatives of HD patients and 22 non-related hospital staff members or subjects vaccinated by their general practitioner). All participants provided informed consent. All subjects received a single intramuscular (deltoid muscle) dose of the monovalent adjuvanted influenza A/California/2009 (H1N1) vaccine commercialized in Belgium (Pandemrix®, GlaxoSmithKline Biologicals, Rixensart, Belgium). Each vaccine dose (0.5 ml) contains 3.75 µg of antigen of split inactivated pandemic (H1N1) 2009 influenza virus and adjuvant system AS03 (10.69 mg of squalene, 11.86 mg of DL-α-tocopherol and 4.86 mg of polysorbate 80). Participants were monitored for the occurrence of any adverse event during the 30 days after vaccination.

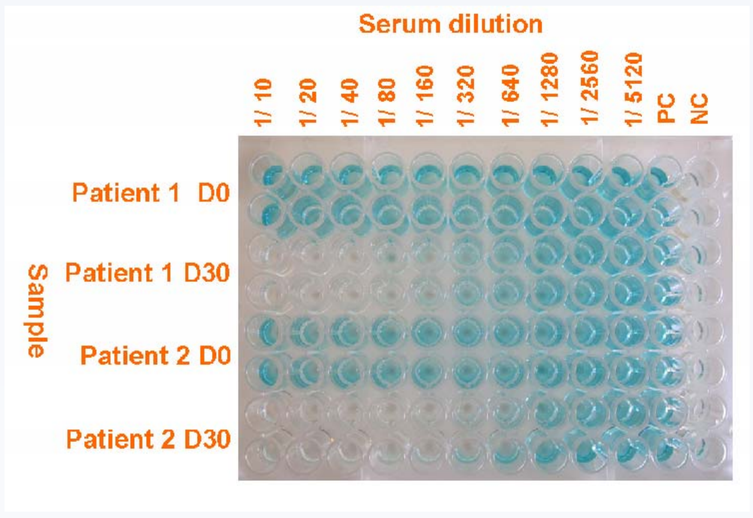

Figure 1. Example of sero-neutralisation test plate after ELISA detection of viral NP protein. Test plate includes 1:2 dilution series of sera from 2 patients (D0 and D30) in duplicate, 8 virus controls (PC) and 8 cell controls (NC).

Sero-neutralisation assay Serum samples were obtained immediately before [Day 0 (D0)] and 30 days after [Day 30 (D30)] vaccination, for antibody titration against the InfluenzaA/California/7/2009 (H1N1) using a seroneutralization assay. The assay is based on the ability of antibodies to inhibit the infection of Madin-Darby canine kidney (MDCK) cell culture by influenza virus, as previously described (1). Briefly, 1:2 serial dilutions of inactivated human serum samples were pre-incubated with a standardized amount of virus (100TCID50) prior to the addition of MDCK cells (25000 cells/well). After overnight incubation, ELISA was used to measure influenza A viral nucleoprotein in infected MDCK cells. Since serum antibodies against influenza virus inhibit the viral infection of MDCK cells, the optical density (OD) results of the ELISA are inversely proportional to the serum antibody concentration. The initial dilution and lowest detection limit of this assay was 1:10. An example of a test plate is shown in figure 1. Suitable control serum samples were included in all analyses, with a post-infection sheep serum sample raised against the A/California/7/2009 (H1N1) strain (FR-188, CDC), and a human serum of convalescent cases of pandemic influenza A (H1N1) and human recipients of A/California/7/2009 (H1N1) (NYMC X179A) vaccine (ref 09/194, NIBSC, London, England) as positive controls. Influenza normal control serum from sheep (FR-49, CDC) was used as negative control.

Samples were tested in duplicate in each assay, and assays were independently repeated once. The titer analyzed was the geometric mean (GM) of these four test results, expressed as the reciprocal of the strongest serum dilution with *OD450* value less than *X*, where *X* = [(average *OD450* of VC wells) – (average *OD450* of CC wells)] / 2 + (average *OD450* of CC cells), with VC = virus control and CC = cell control (1). Samples without detectable antibody activity were assigned the titer of half the assay detection limit (1:5). Titers were expressed as the reciprocal of the dilution.

Statistical analysis GM titers were determined at subject level by individual GM of 4 test results at each time point and at group level by GM of all subject GM titers. In addition, individual and group level GM titer ratios (GMTR) (GM titer D30 / D0) were determined to measure the factor increase in GM titers. For each variable, the result is expressed as point estimate with corresponding 95% confidence intervals (95%CI). Seroconversion was defined as an increase in GM titer by a factor 4 or more. Student T tests were used to compare group GM titers at D0 and D30 and GMTR. The proportion of seroconversions in each group at each time point was compared by Fisher's exact tests. Statistical significance level was set at $p<0.05$. The primary endpoint was the proportion of subjects reaching seroconversion in each group. Secondary endpoints were GM titers and GMTR reached in both groups and the occurrence of severe adverse reactions.

Results

Immunogenicity of the Pandemrix vaccine Group level results are presented in Table 1. The GM titer ratio for the healthy control group was 38 (23-62). For the dialysed patients, the GM titer ratio was 11 (8-17) and for the group of renal transplanted patients, the GM titer ratio was 5 (3-6). Differences in GM titer ratio between the healthy control group and each group of immunodepressed patients were significant ($p<0,05$; Student T test). Thirty out of 32 healthy participants (94%) had at least four-fold increases in SN titers between day 0 and day 30 (seroconversion). Among dialysed participants, 64 out of 106 (60%) had at least four-fold increases in SN titers between day 0 and day 30, and among renal transplanted patients, 49 out of 112 participants (44%) seroconverted between day 0 and day 30. Differences in seroconversion rates between the healthy control group and each group of immunodepressed patients were significant ($p<0,05$; fisher exact test).

	GMT D0	GMT D30	GMTR	% seroconversion
CONTROLS (n=32)	10 (6.0-16)	374 (216-648)	38 (23-62)	94
HEMODIALYSED (n=106)	8.1 (7.0-9.5)	76.3 (51.5-113)	11 (7.5-17)	60
RENAL TRANSPLANTED (n=112)	11 (8.0-14)	49 (34-70)	5 (3-6)	44

Table 1. Group level geometric mean titers (GMT), geometric mean titer ratios (GMTR) (D30-D0) and % seroconversion (4-fold increase in titer) for controls, hemodialysed and renal transplanted patients.

Adverse effects With the exception of two HD patients, who presented moderate local pain at the site of injection, no other side effects associated with the vaccine were observed in patients or controls.

Conclusion

These results suggest that only 60% of dialysed individuals developed sufficient neutralizing antibody titers after immunization with this vaccine, in copmparison to 94% of the controls. As for renal transplanted patients, only 44% of the patients developed sufficient neutralizing antibody titers after vaccination. Alternative vaccines, dosing, adjuvants or schedule strategies are needed to achieve effective immunization of these vulnerable populations.

References

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ISP-WIV | Rue Juliette Wytsmanstraat 14 | 1050 Brussels | Belgium
T +32 2 642 55 96 | F +32 2 642 50 01 | e-mail: anneleen.hombrouck@wiv-isp.be | site web: www.wiv-isp.be