

Persistence and avidity maturation of antibodies to A(H1N1)pdm09 in healthcare workers following repeated annual vaccinations



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ARTICLE INFO

Article history:

Received 16 January 2015

Received in revised form 12 May 2015

Accepted 27 May 2015

Available online 7 June 2015

Keywords:

Influenza

Repeated vaccination

Long-term antibody persistence

Healthcare workers

Avidity

ABSTRACT

Healthcare workers are at increased risk of influenza infection through direct patient care, particularly during the early stages of a pandemic. Although influenza vaccination is widely recommended in Healthcare workers, data on long-term immunogenicity of vaccination in healthcare workers are lacking.

The present study was designed to assess the persistence of the humoral response after pandemic vaccination as well as the impact of repeated annual vaccination in healthcare workers ($n = 24$).

Pandemic influenza vaccination resulted in a significant increase in haemagglutination inhibition (HI) antibody titers with 93–100% of subjects achieving protective titers 21-days post each of the three annual vaccinations. Seroprotective antibodies measured by HI, microneutralization and single radial hemolysis assays were present in 77–94% of healthcare workers 6 months post-vaccination. Repeated vaccination resulted in an increased duration of seroprotective antibodies with seroprotective titers increasing from 35–62% 12 months after 2009 pandemic vaccination to 50–75% 12 months after 2010 vaccination. Furthermore, repeated annual vaccination augmented the avidity of influenza-specific IgG antibodies.

In conclusion, we have shown that A(H1N1)pdm09 vaccination induces high seroprotective titers that persist for at least 6 months. We demonstrate that repeated vaccination is beneficial to healthcare workers and results in further avidity maturation of vaccine-induced antibodies.

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1. Introduction

The first cases of human infection with a novel influenza A H1N1 virus, A(H1N1)pdm09, occurred in April 2009 in Mexico and North America. The virus rapidly spread globally with an influenza pandemic declared on June 11, 2009 [1]. The A(H1N1)pdm09 virus had a classical swine H1 haemagglutinin (HA); antigenically distinct from recently circulating seasonal H1N1 strains [2]. An estimated 1 million people in Norway were infected by the A(H1N1)pdm09 virus in 2009 [3]. The pandemic was considered mild and the

average case fatality rate of 0.15–0.25% suggests that there was some degree of pre-existing immunity in the population [4]. Healthcare workers (HCWs) and persons in risk groups were initially prioritized for vaccination and 2.2 million people (45% of the population) were vaccinated in Norway during the pandemic [5]. In 2010, the A(H1N1)pdm09 virus entered a post-pandemic period and continued circulating as a seasonal virus. Consequently, seasonal trivalent influenza (TIV) vaccines from 2010 include the A(H1N1)pdm09 virus as the H1N1 strain [6].

To address the decline in serum antibody titers that may occur within a year of vaccination, annual re-vaccination is required to increase the effectiveness of influenza vaccines. However, the value of repeated annual vaccination still remains a subject of discussion as clinical studies have shown mixed results [7–11]. Lower immunogenicity of seasonal influenza vaccines has been occasionally reported in previously vaccinated adults [7–9]. Other studies reported similar or better effectiveness of influenza vaccination in

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previously primed individuals [10,11]. Furthermore, higher pre-vaccination antibodies were reported in previously vaccinated individuals than those with no previous vaccination [11,12].

A single dose of A(H1N1)pdm09 vaccine induced protective Haemagglutination Inhibition antibody titers in adults [13–15]. However, limited data are available on antibody persistence and antibody avidity maturation after repeated annual vaccination with A(H1N1)pdm09-containing vaccines. We therefore conducted this clinical study to evaluate the kinetics and quality of the antibody response in HCWs after pandemic vaccination in 2009 and subsequent immunization with trivalent seasonal A(H1N1)pdm09-containing vaccines in 2010 and 2011.

2. Materials and methods

2.1. Study protocol

We conducted a clinical study to evaluate the immunogenicity of pandemic influenza vaccination in HCWs. In October 2009, HCWs were vaccinated at the hospital with pandemic vaccine. Of these vaccinated HCWs, 24 HCWs (aged 21–49) working on the infectious disease ward were followed to investigate the long-term immunogenicity of the A(H1N1)pdm09 vaccine. Furthermore, the effect of revaccination was evaluated. The inclusion and exclusion criteria for this study are described elsewhere [16]. All participants provided written informed consent before inclusion in the study, which had ethical and regulatory approval (ClinicalTrials.gov NOT01003288).

The pandemic vaccine (Pandemrix®) was a monovalent split-virus vaccine (3.75 µg HA of A/California/07/2009-like virus(H1N1) adjuvanted with AS03 (GSK, Belgium). In 2010 and 2011, the HCWs were vaccinated, with seasonal TIV (Influvac®). The 2010–2011 and 2011–2012 TIV contained 15 µg HA of A/California/7/2009 (H1N1)-like, A/Perth/16/2009 (H3N2)-like and B/Brisbane/60/2008-like strains.

Serum samples were collected at 10 time-points: at recruitment in 2009 (days 0 & 21, 3 and 6 months post-pandemic-vaccination. Further serum samples were collected on subsequent vaccinations: 12-months post 2009 vaccination, 21-days and 6-months after both 2010 and 2011 vaccinations (Fig. 1). All serum samples were aliquoted and stored at –80 °C before use in the serological assays.

2.2. Haemagglutination inhibition (HI) assay

Serum samples were treated with receptor destroying enzyme and run in the HI using A(H1N1)pdm09 virus as previously described [16]. Seroprotection was defined as an HI titer ≥ 40 and titers < 10 were assigned a value of 5 for calculation purposes.

2.3. Virus microneutralization assay

The microneutralization assay was performed using A/California/7/2009 like-virus yielding $2 \times 10^{3.3}$ TCID₅₀/ml as previously described [17]. The highest serum dilution that neutralized $\geq 50\%$ of the wells was determined. Sera with titers ≥ 10 were considered positive and antibody titers < 10 were assigned a value of 5

for calculation purposes. A MN titer ≥ 80 was considered seroprotective [18].

2.4. Single radial haemolysis (SRH) assay

SRH was performed according to previously described procedures using whole inactivated H1N1-virus [19] based on a standardised method [20]. A haemolytic zone with area ≤ 4 mm² was considered seronegative, areas of 4–24 mm² were considered positive but not protective, and ≥ 25 mm² was considered as protective according to the CHMP guidelines [21].

2.5. Specific IgG ELISA

Sera were evaluated for IgG antibodies [22]. The plates were coated with influenza whole A(H1N1)pdm09 virus (2 µg/ml) or capture IgG antibody (1 µg/ml). The antibody concentrations were calculated using the IgG standard and linear regression of the log-transformed readings.

2.6. Avidity ELISA

Sera were evaluated for avidity of antibodies against influenza HA1 (A/California/06/2009(H1N1)) 6XHis tagged (eEnzyme, USA) [23]. Sera were first diluted to the appropriate Optical Density (OD) of 0.7 ± 0.3 in a direct ELISA and 1.5 M Sodium thiocyanate (NaSCN) was added 1 h after the sera, followed by 1 h incubation. The percentage antibody remaining after treatment of the sera with 1.5 M NaSCN was calculated as: $(OD_{450} \text{ treated serum} / OD_{450} \text{ untreated serum}) \times 100\%$.

2.7. Statistical analysis

Data analyses were performed using Graphpad Prism version 5. One-way ANOVA with Tukey test was used for multiple comparisons. Correlation between the serological HI and MN titers was performed using Spearman rank test. A *p*-value < 0.05 was considered statistically significant.

3. Results

3.1. Influenza A(H1N1)pdm09 vaccination provided long-term protection in HCWs

Prior to the 2009 pandemic vaccination, 42% of HCWs were seropositive (GMT16). Upon vaccination, there was a significant increase in HI titers with the CHMP licensing criteria for seroconversion and seroprotection (HI titers ≥ 40) being met by all the HCWs 21-days post-vaccination ($p < 0.01$) (GMT711) (Fig. 2A), a 44-fold increase (Fig. 2B). Antibodies persisted at 3- and 6-months post-vaccination, meeting the CHMP criteria for seroprotection in 92% and 85% of HCWs, respectively (Fig. 2A and C). Vaccination with 2010 TIV in the subsequent season boosted the HI titers at 21-days post-vaccination (GMT232) with all but one individual achieving seroprotective HI titers. No significant difference in HI titers at 21-days in 2009 were observed from those at 21-days after 2010

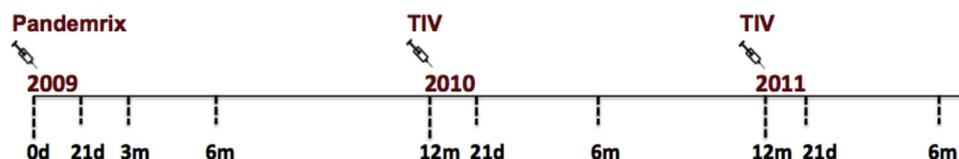


Fig. 1. A schematic of the clinical trial. Healthcare workers received the Pandemrix vaccine in 2009 and were revaccinated annually in 2010 and 2011 with the trivalent inactivated vaccine. Serum samples were collected on 10 different time points. 0d, 0 days; 21d, 21 days; 3m, 3 months; 6m, 6 months; 12m, 12 months post-vaccination.

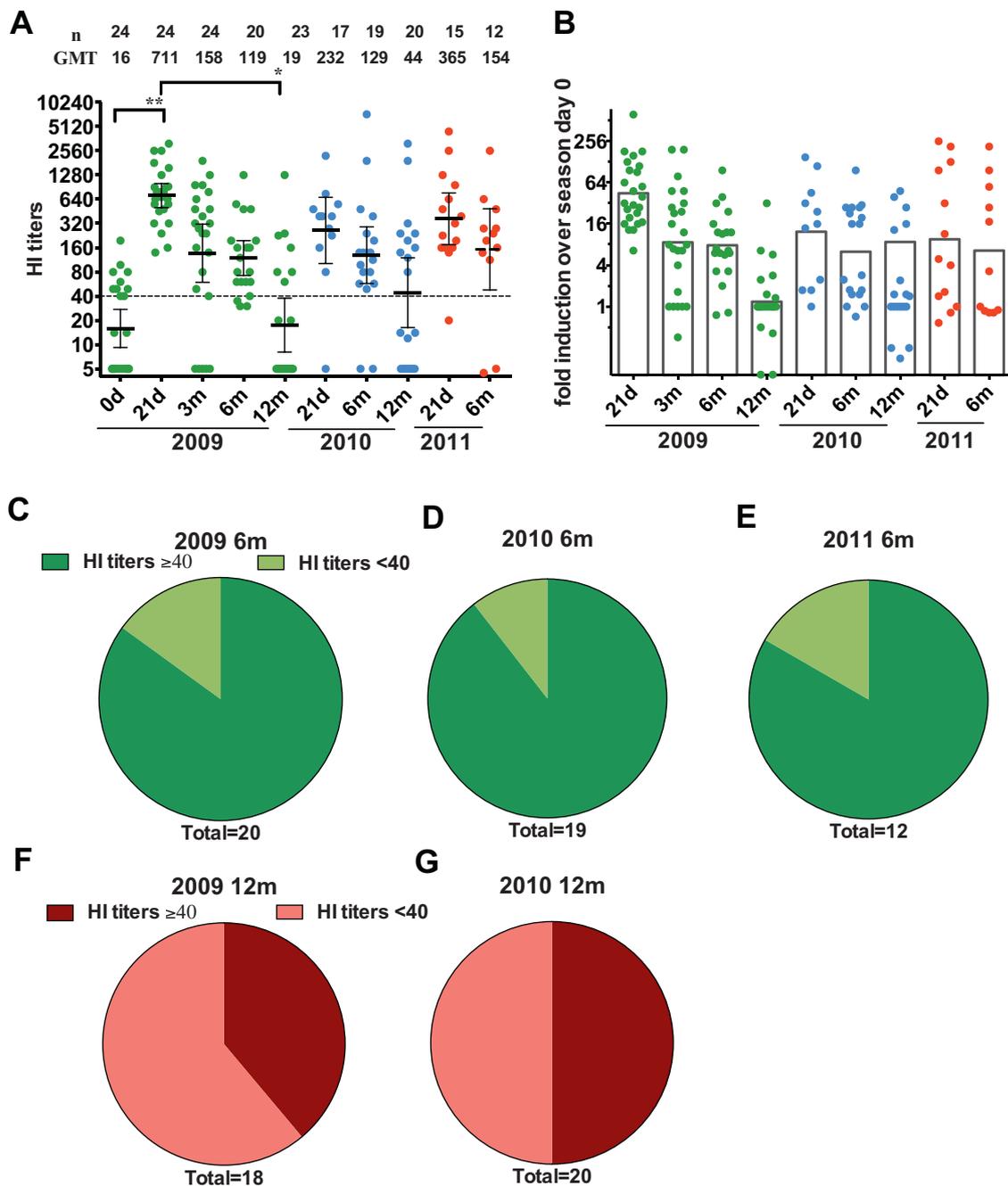


Fig. 2. Hemagglutination inhibition response. (A) HI titers against influenza A (H1N1)pdm09 virus at all 10 time-points. The dotted line indicates a titer of 40, which is considered protective. The number of samples and Geometric Mean Titers are shown as numbers above the plots for each time-point. * Indicates statistically significant differences in responses ($p < 0.05$), measured with One-way ANOVA with Tukey's multiple comparison test. (B) Fold-induction of HI titers over pre-vaccination titers for each of the 3 seasons. The columns indicate the geometric mean fold of induction. Pie diagrams with proportion of HCWs who maintained HI titers ≥ 40 at 6 months (C–E) and 12 months (F and G) post vaccination.

and 2011 vaccination, with 94% and 93% of HCWs seroprotected, respectively. Interestingly, the HI antibodies did not wane quickly over time but persisted for at least 6-months after 2010 vaccination, with 94% maintaining seroprotective titers (Fig. 2D). Similar kinetics of the antibody responses were observed after vaccination with 2011 TIV. 2011 vaccination boosted HI titers in all individuals by day 21 (GMT365) with the seroprotective titers persisting 6 months post-vaccination in 83% of HCWs (Fig. 2E). Whilst antibodies persisted for 6 months post-vaccination, the persistence rate declined by 12 months. At 12 months after 2009 pandemic vaccination, the HI titers had declined significantly ($p = 0.01$) in comparison to day 21 titers, although 8 HCWs (35%) had persisting protective

antibody titers (HI titers ≥ 40) (Fig. 2F). However, following revaccination in 2010, antibody persistence at 12 months had improved to 50% (Fig. 1G).

3.2. Microneutralization antibodies correlated with HI response

Microneutralization titers were examined in the 13 individuals who provided consecutive samples throughout the study. Pre-pandemic vaccination, only one (8%) of the 13 subjects tested had neutralizing antibodies (titer ≥ 80). Post-pandemic-vaccination, a significant increase was observed in MN titers ($p < 0.01$) at day 21 with all subjects having protective titers (GMT906) (Fig. 3A). Three

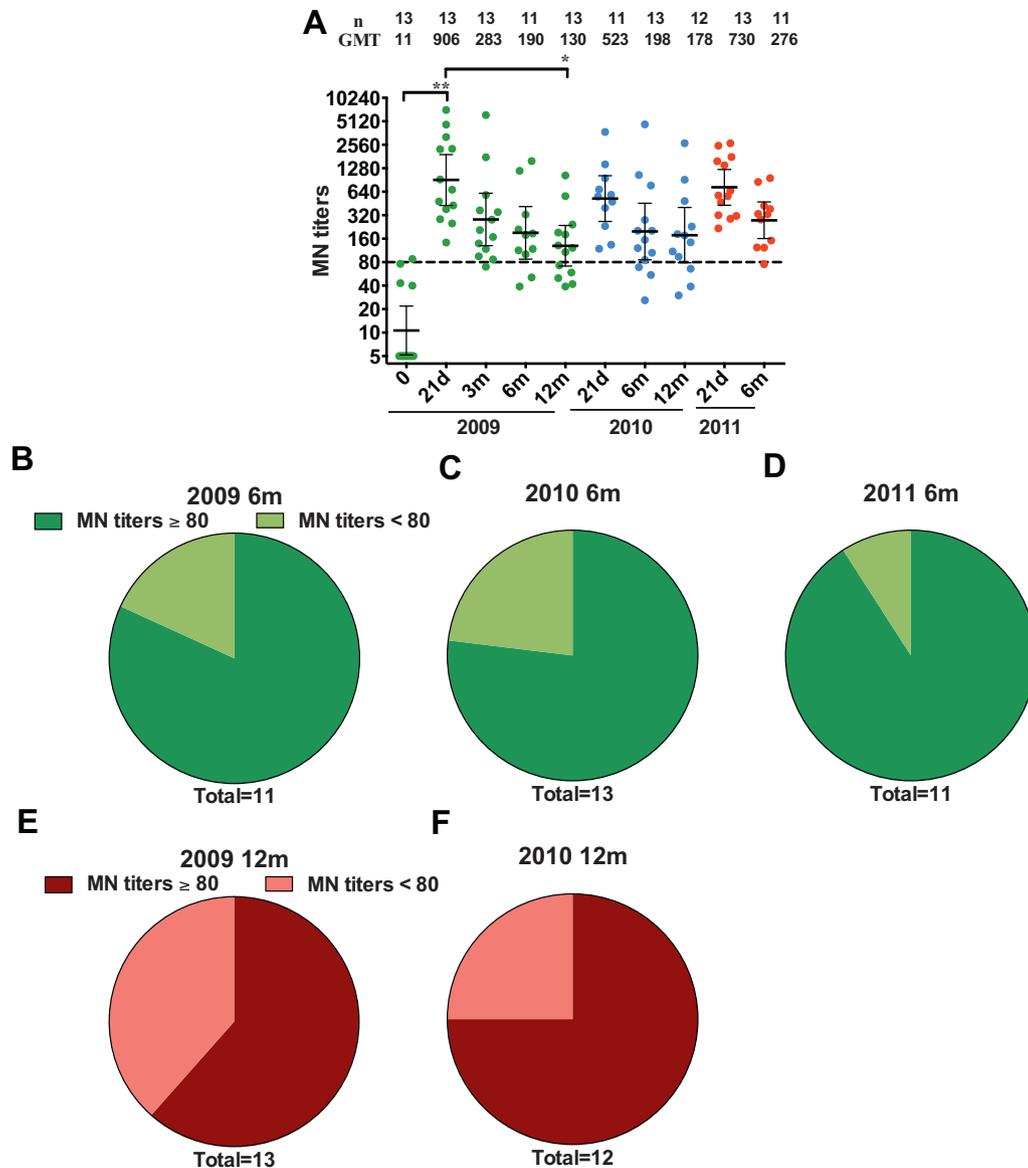


Fig. 3. The serum virus microneutralization response. (A) MN titers following repeated annual vaccination. The dotted line indicates a titer of 80, considered protective. Pie diagrams with proportion of HCWs who maintained protective MN titers ≥ 80 at 6 months (B–D) and 12 months (E and F) post vaccination. * Indicates statistical significance $p < 0.05$; ** $p < 0.01$ measured with One-way ANOVA with Tukey's multiple comparison test.

and six-months post-vaccination, the neutralizing antibodies persisted in 92% (GMT283) and 82% (GMT190) HCWs (Fig. 3A and B), respectively. At 12 months post-pandemic-vaccination, the neutralizing antibodies decreased significantly (GMT130) compared to 21-days post-vaccination (GMT906) ($p < 0.05$). However, the neutralizing antibodies remained above the protective titer in 62% of subjects (Fig. 3E).

2010 vaccination with TIV boosted the response with all subjects achieving protective MN titers by 21-days post-vaccination. While seroprotective HI titers were present in 50% of subjects at 12-months, the MN antibodies persisted long term at 6 and 12-months post-2010 vaccination in 77% and 75% of subjects, respectively (Fig. 3C and F). Subsequent vaccination in 2011 resulted in similar kinetics of the antibody responses with 100% achieving MN titers ≥ 80 . These protective antibodies persisted 6-months after 2011 vaccination in 91% of subjects (Fig. 3D). There was a significant correlation between MN and HI titers at all time-points post-vaccination (Spearman $r \geq 0.50$, $p < 0.05$) (Supplementary Fig. 1).

3.3. SRH antibodies support HI and MN measurements

Pre-pandemic vaccination, 9 subjects (39%) had no SRH antibodies. Seroprotective titers with zone areas $\geq 25 \text{ mm}^2$ were detected in 4 subjects (17.4%). By 21-days, all subjects had achieved protective SRH titers ($p < 0.0001$) (GMT79.8) (Fig. 4A), geometric mean increase of 6.6 (Fig. 4B). At 3-months post-2009-vaccination, protective titers were maintained by 87.5% of HCWs. The protective SRH titers persisted in 80% and 59% of subjects at 6- and 12-months post-2009 vaccination, respectively (Fig. 4C and E). Upon 2010 TIV-vaccination, the seroprotection rate of 88.2% assessed by SRH at day 21 was similar to that measured by HI at 94% (Fig. 4A). The GMT increased 2.2 fold over titers observed 12-months post-2009-vaccination. The protective antibody titers persisted for at least 6-months after 2010-vaccination in 68.8% of subjects (Fig. 4D). The long-term seroprotection rate 12-months post-vaccination increased from 59% after 2009-vaccination to 73.3% after 2010-vaccination (Fig. 4F). Revaccination in 2011 resulted in boosting

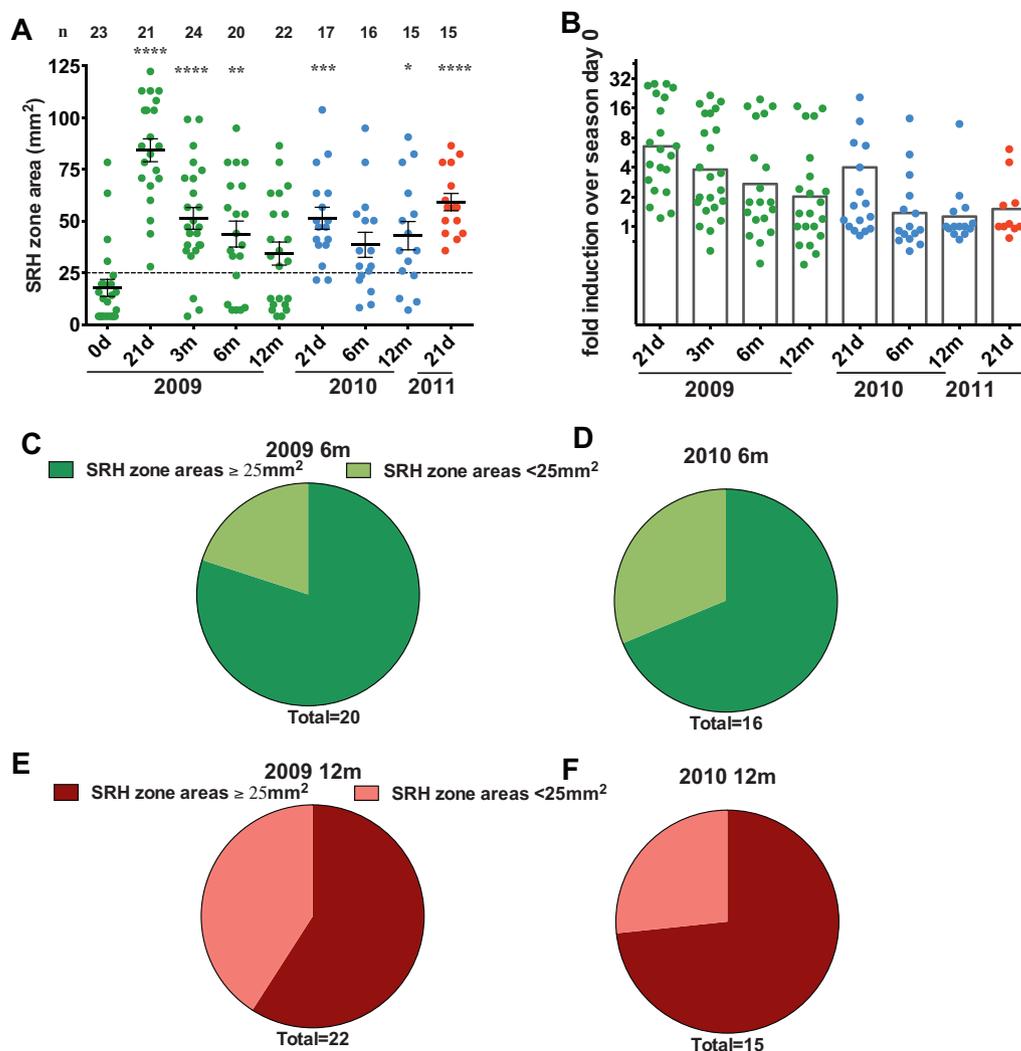


Fig. 4. Single radial hemolysis antibody response. (A) SRH titers against whole inactivated A (H1N1)pdm09 virus following 3 annual vaccinations in HCWs. The dotted line indicates SRH areas $\geq 25\text{mm}^2$, defined as seroprotective. (B) Fold-induction of SRH titers over titers at time of vaccination for each of the 3 seasons. The columns indicated the geometric mean fold of induction. Pie diagrams with proportion of HCWs who maintained SRH areas $\geq 25\text{mm}^2$ at 6 months (C and D) and 12 months (E and F) post vaccination. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, **** $p < 0.0001$ measured with One-way ANOVA with Tukey's multiple comparison test.

of the SRH antibodies, with all subjects achieving seroprotective titers by day 21, 1.5 fold increase over 12months in 2011.

The kinetics of the MN antibody titers followed those of the HI response post-vaccination, although MN titers were generally higher (Fig. 5A). Similar to the HI and MN antibodies, the highest SRH titers were observed at day 21 after 2009 vaccination. We evaluated the kinetics of the SRH response in relation to that of HI and MN response after vaccination. The SRH antibodies correlated with the HI titers (Fig. 5B) and the MN titers (Fig. 5C) after all 3 vaccinations (Supplementary Figs. 2 and 3). However, when the antibody titers were at their highest, 21-days post-2009-vaccination, no significant correlation was observed between SRH titers and HI or MN titers despite 100% seroprotection rates in all serological assays.

Repeated vaccination with A(H1N1)pdm09 resulted in enhancement of IgG antibody avidity but not concentration.

The kinetics of the serum IgG antibody responses to A(H1N1)pdm09 were analyzed by ELISA with whole virus as the antigen. The A(H1N1)pdm09-specific IgG antibodies were present in all subjects before pandemic vaccination and increased at 21-days post pandemic vaccination. Furthermore, seasonal TIV vaccinations in 2010 and 2011 did not result in significant changes in the A(H1N1)pdm09-specific IgG concentrations (Fig. 6A).

To assess the effect of repeated vaccination on antibody avidity maturation, we measured the strength of binding between IgG antibodies and the major surface protein, HA1 after each vaccination with A(H1N1)pdm09-containing vaccines. Untreated sera were compared to those treated with 1.5 M NaSCN, and the percentage of bound IgG antibodies remaining after 1.5 M NaSCN treatment was calculated (yielding an 81–97% reduction in antibody binding). Pre-pandemic vaccination, 3% of IgG antibodies were bound after 1.5 M NaSCN treatment (Fig. 6B). Pandemic vaccination resulted in an increase in the IgG avidity with 7% of the antibodies bound at day 21, which was a 1.43 fold increase from pre-vaccination. The mean values of antibody avidity declined slightly by 3 and 6 months after pandemic vaccination. However, 2010 vaccination resulted in the increase in antibody avidity with 14% of IgG antibodies remaining bound at 21-days post-vaccination; a mean induction of 3.89 over pre-pandemic levels. Interestingly, re-stimulation of the immune system with 2011 vaccination, 12 months later, resulted in further increase in the avidity of the IgG antibodies. The highest antibody avidity was observed 21-days post-2011-vaccination with a mean 19% of IgG antibodies remaining bound ($p < 0.05$) (Fig. 6B), mean induction of 4.39 fold against pre-pandemic (Fig. 6C). The percentage of bound antibodies 21-days after the third vaccination,

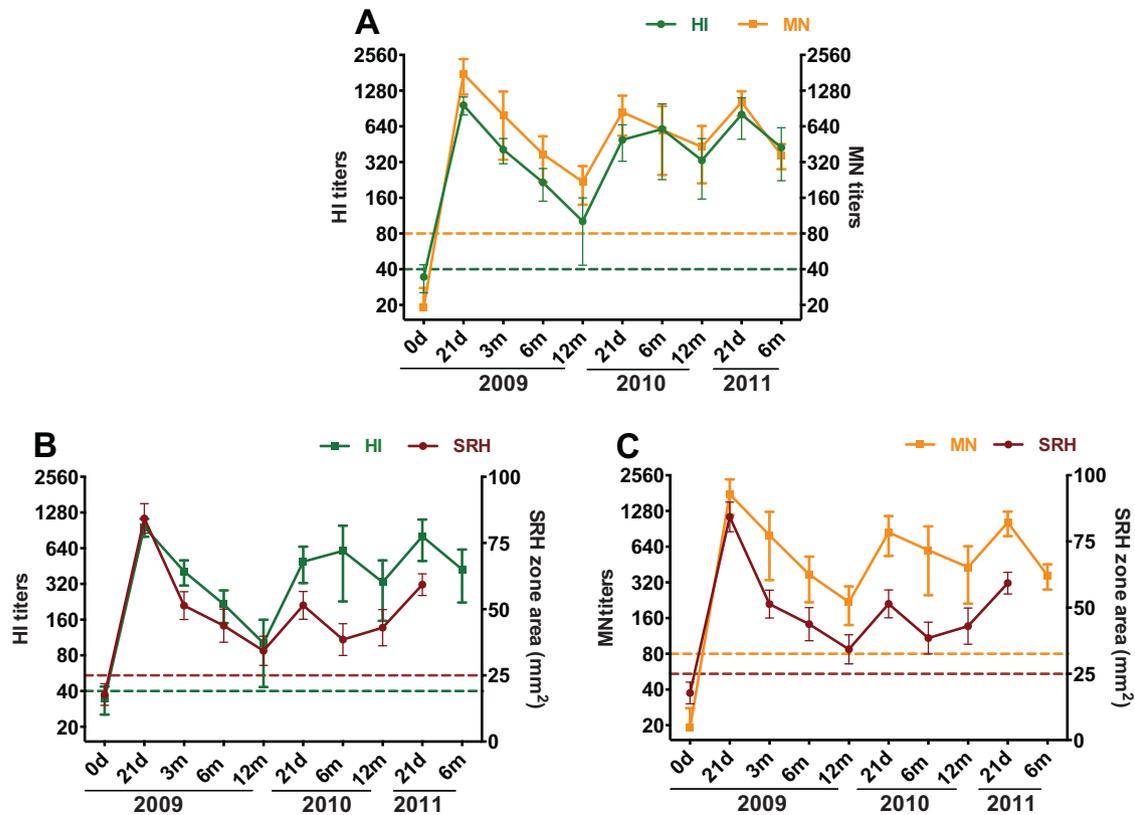


Fig. 5. Kinetics of the HI, MN and SRH response. (A) The kinetics of the total MN titers follow the kinetics of the total HI titers. (B) Kinetics of the HI and SRH titers. (C) Kinetics of the MN and SRH titers. Each symbol represents the GMT of all the titers at each time-point \pm SEM (bars). The dotted lines indicated the seroprotective titers for the 3 serological assays; HI (green), MN (orange) and SRH (red).

in 2011, was significantly higher than those observed 21-days post-pandemic vaccination ($p < 0.05$). These results suggest that repeated vaccination results in avidity maturation of antibodies that may contribute to long-term protection against influenza.

4. Discussion

There are limited numbers of studies investigating the effect of yearly influenza vaccination on the immune response in HCWs, who are recommended for annual influenza vaccination. In this study, we evaluated the antibody response against influenza A(H1N1)pdm09 virus to assess the antibody persistence after repeated vaccinations in HCWs. At baseline, HI antibody titers were observed in 42% of subjects. This could be attributed to pre-existing antibodies as a result of previous or recent subclinical infection with the pandemic H1N1 virus, during their work as frontline healthcare workers at the infectious disease ward with continuous exposure to patients hospitalized with influenza infection. Notably, the start of pandemic vaccination coincided with the increase in the number of new hospitalizations while consultations for influenza-like-illnesses peaked 1–2 weeks later [3]. Receipt of recent (2005–2009) seasonal vaccination did not offer protection against A(H1N1)pdm09 as the virus was antigenically distinct from recently circulating seasonal H1N1 strains [24]. However, cross-reactive antibodies to A(H1N1)pdm09 were detected in persons >65 years old. Conversely, these antibodies were absent in children and young adults [25,26]. Serum antibodies from elderly adults (>60 years old), post-vaccination as well as post-infection, demonstrated superior avidity than those from younger adults [27,28]. This may account for the age-related morbidity and mortality during the 2009 pandemic.

This study examines the effect of repeated annual vaccination on the functional capability of induced antibodies and maturation of immune response as measured by avidity. We found that the kinetics of the IgG avidity did not follow that of the serum A(H1N1)pdm09-specific IgG concentrations. Of note, IgG ELISA quantifies IgG antibodies directed to the whole virus, while the avidity measures antibodies that bind epitopes spanning the receptor binding site on the HA globular head domain.

In our study, the adjuvant may have influenced the HI titers, MN titers and avidity of the antibodies after the first vaccination only. AS03 adjuvanted pandemic H1N1 vaccine has been previously shown to have superior immunogenicity to the unadjuvanted pandemic H1N1 vaccine. AS03 induced superior antigen-specific antibody levels and immune memory [29]. Despite no significant changes in the influenza-specific IgG concentrations after 2010 and 2011 vaccinations, repeated vaccination with TIV containing A(H1N1)pdm09 augmented the avidity of the HA-specific IgG antibodies. The avidity was lowest at the time of the first vaccination (2009) and increased after each subsequent vaccination, with the highest avidity being observed after the third vaccination. The majority of the vaccinees had exceeded a minimum threshold for protective antibodies after the first vaccination, further increases in antibody avidity were not associated with improvements in antibody concentrations or neutralizing capacity. This suggests that maintenance of a low number of high avidity antibodies is sufficient for protection [28,30]. High titers of low avidity antibodies in infected individuals were associated with severe A(H1N1)pdm09 disease [31]. On subsequent seasonal vaccination, whereby the vaccines did not contain an adjuvant, the increase in antibody avidity may be attributed to the recall of long-term memory B cells generated after the first vaccination (2009) that re-enter germinal centers and undergo further maturation and differentiation

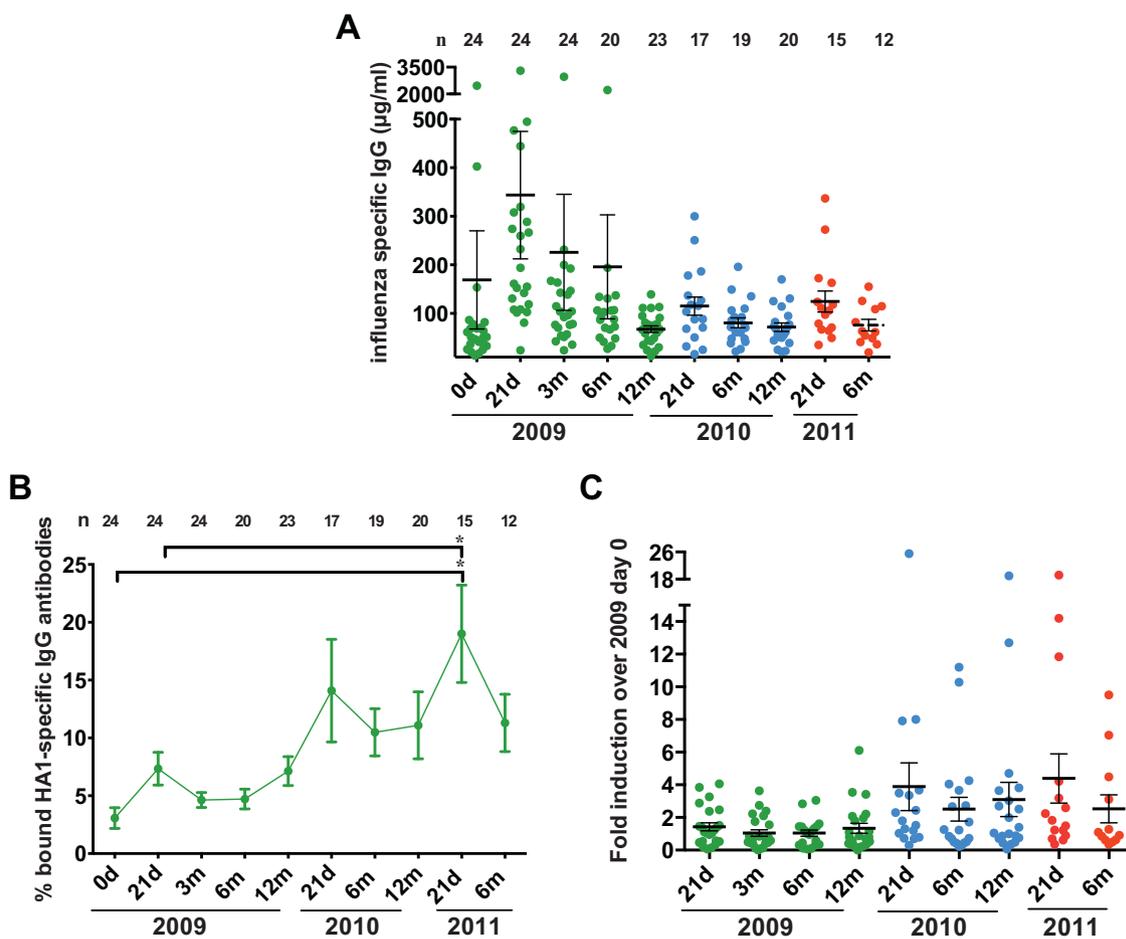


Fig. 6. Influenza specific IgG concentration and avidity. (A) The concentrations of (H1N1)pdm09 whole virus specific IgG in serum pre-vaccination and following 3 annual vaccinations. (B) Standardized sera were treated with 1.5 M NaSCN and the percentage of HA1-specific IgG antibodies remaining bound after 1.5 nM NaSCN treatment was measured as (absorbance of treated samples/absorbance of untreated samples) \times 100%. Each symbol represents the mean of all the titers at each time-point \pm SEM (bars). (C) Fold induction of % bound HA1-specific antibodies over pre-vaccination at 2009 0d. * Indicates statistical significance $p < 0.05$ measured with One-way ANOVA with Tukey's multiple comparison test.

resulting in generation of antibodies with increasing avidity [32]. Avidity may be an indicator of protection and could be used as a surrogate for priming of immunological memory, as it is determined by the immunoglobulin gene sequences as a result of somatic hypermutation and selection [33]. Furthermore, there is an indication of a link between avidity and protection in that seroprotective antibody titres persisted longer with each subsequent vaccination in parallel with the increase in antibody avidity. The potential of antibody avidity as a measure of protection or vaccine efficacy needs to be investigated further following influenza vaccination as well as natural infection.

Other than viral neutralization, high avidity antibodies may play roles important for anti-viral immunity such as complement-dependent cytotoxicity or antibody-dependent cell-mediated cytotoxicity [34–36].

We observed seroprotective HI titers in all HCWs 21-days after a single dose of adjuvanted pandemic vaccine. Lower seroprotective titers have been reported for HCWs in Hong Kong (54%) and Japan (38%) after one dose of un-adjuvanted A(H1N1)pdm09 vaccine [37,38]. These inferior responses may be attributed to lack of adjuvant in the vaccines administered in these studies. In the Netherlands, 80% of HCWs achieved protective HI titers with MF59 adjuvanted vaccine [39]. The oil-in-water emulsion adjuvants, MF59 and AS03, are known to enhance antibody responses after H1N1 and H5N1 vaccination and also allow considerable dose

sparing [40–43]. Neutralizing antibody responses as determined by MN and HI assays have a significant role in prevention and clearance of infection. The MN and HI antibody titers generally correlate, with the MN assay exhibiting better sensitivity [25]. However, no correlates of protection have been established for MN antibody although MN titers of 80 have been proposed as protective [44]. All subjects in our study achieved the proposed protective MN 21-days after each vaccination. A significant correlation was identified between MN titers and the magnitude of the HI response at all time points in the three seasons, in agreement with previous studies [18,25]. This correlation between HI and MN assays highlights that MN can be used for evaluation of the immunogenicity of influenza vaccines. Furthermore, we show that the influenza-specific antibodies inducing complement-mediated haemolysis correlate strongly with neutralizing antibodies measured by HI and MN assays, suggesting that the SRH antibodies are largely neutralizing.

Studies suggest that antibody responses in previously vaccinated subjects were lower than in subjects with no prior vaccination [12,39]. This may be due to residual protection resulting in attenuated antibody responses [45]. In agreement, higher baseline HI titers in previously vaccinated subjects were reported to be associated with lower immune response. However, unique to our study, we show by three serological assays that repeated annual vaccination over 3 years did not result in attenuated antibody responses to A(H1N1)pdm09 but that protective antibodies

are elicited after each vaccination and persist over time. The HI, MN and SRH antibodies elicited by the vaccinations were persistent, increasing and sustained for at least 6 months following each vaccination in most subjects (77–94%). HI antibodies persisted in 35% of subjects 12-months after pandemic vaccination while 50% subjects maintained seroprotective HI titers at 12 months in 2010. The proportion of HCWs with seroprotective titers detected by in MN and SRH assays increased from 59 to 62% 12-months after pandemic vaccination to 73–75% 12-months after 2010-vaccination. The lower antibody titers at 12-months may not necessarily mean that the HCW were no longer protected, as factors other than antibody titers may be important in long-term protection. Importantly, vaccination induces long-lived memory B cells that can be reactivated upon subsequent infection to produce high-avidity antibodies [46,47].

There have been concerns that repeated annual influenza vaccination with inactivated vaccines might increase the severity of subsequent influenza infection, as TIV induces ineffective cross-protective CD8 T cell responses, which are boosted by natural infection [48,49]. However, a study reported a similar influenza-specific CD8 T-cell memory response at 8–10 months post-vaccination and infection [49]. Ideally, further studies are required to study the cross-protective responses induced by vaccination. Another concern is that TIV induces poor mucosal immune responses, which may negatively affect vaccine effectiveness [50]. However, given the favorable and high seroprotective rates that persist long term and the increase in antibody avidity with repeated vaccinations in our study, promoting enthusiasm for annual influenza vaccination among HCWs should be encouraged. Mandatory vaccination policies as well as improved vaccine access increase vaccine uptake in HCWs [51–53]. High rates of influenza vaccination in HCWs will be of great benefit to the HCW, the patients and the communities in which they live. Importantly, vaccination will likely reduce the number of influenza-related infections and deaths.

Contributors

S.E performed the experiments and was involved in interpreting the data. S.M.T was involved in interpreting the data and prepared the manuscript. A.J-L contributed to the daily supervision of the study, analysis and interpretation of results. KH contributed to experimental design and interpretation of results. R.J.C, K.A.B and E.M contributed to the study design, protocol design and interpretation of the results. All authors critically reviewed the manuscript and approved the final article.

Conflict of interest statement

None.

Acknowledgements

We thank the Healthcare workers at HUH for participating in this study. We thank Dr. Steinar Skrede, Dr.Per Espen Akslesen, Prof. Haakon Sjursen and the nurses at the Bergen Clinical Vaccine Consortium, Haukeland University Hospital, Bergen Norway. Aram Afsar from Health Protection England, UK for discussion. The study was funded intramurally by the Influenza Centre at the University of Bergen and through funding from the Norwegian Directorate of Health. The Influenza Centre is funded by the Ministry of Health and Care Services, Norway, the Norwegian Research Council Globvac programme (220670/H10), the European Union (Univax 601738), EU IMI FLUCOP (115672), Helse Vest and the K.G. Jebsen Centre for Influenza Vaccines.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.vaccine.2015.05.081>

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