Influenza A(H1N1)pdm09 antibodies after pandemic and trivalent seasonal influenza vaccination as well as natural infection in November 2010 in Hamburg, Germany

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The 2009 influenza pandemic has introduced the new re-assorted influenza A(H1N1)pdm09 virus which recirculated during the 2010/11 influenza season. Before that season, it was possible to acquire protective immunity either by pandemic or seasonal influenza vaccination against influenza A(H1N1)pdm09 or by natural infection. To obtain data on vaccination coverage and antibody levels in a reference population and to calculate whether or not the herd immunity threshold (HIT, calculated as 33% given an R_0 of 1.5) was reached at the beginning of the 2010/11 season we performed a seroprevalence study in November 2010 in Hamburg, Germany. Antibody titres were assessed applying a haemagglutination inhibition test. Vaccination coverage was very low: 14% for pandemic and 11% for seasonal 2010/11 vaccinations. Even in those with underlying risk factors, vaccination coverage was not much higher: 17% for both vaccines. Serological analysis revealed antibody titres of ≥1:10 in 135 of 352 (38%) and of ≥1:40 in 61 of 352 study participants (17%). Specific antibodies were measurable in 26% of those without history of vaccination or natural infection, indicating a high proportion of subclinical and mild influenza disease. Nevertheless, the HIT was not reached, leaving the majority of the population susceptible to influenza A(H1N1)pdm09 and its potential complications.

Introduction

In April 2009, a new re-assorted influenza A virus emerged causing the influenza A(H1N1)pdm09 pandemic [1]. In Germany, a monovalent AS03-adjuvanted vaccine against the pandemic influenza A(H1N1)pdm09 (Pandemrix) has been available since October 2009 and was recommended to persons at risk for severe disease but was also offered to anyone who wanted to be vaccinated for maximal personal protection. In addition, a trivalent seasonal influenza vaccine was available as in previous years [2]. The end of the pandemic was declared in August 2010, and haemagglutinin and neuraminidase antigens H1 and N1 of the pandemic strain were integrated into the 2010/11 trivalent seasonal influenza vaccines, which became available in Germany in September 2010. Therefore, antibodies to influenza A(H1N1)pdm09 detected in November 2010, before the start of the influenza season 2010/11 were a consequence either of vaccination with the pandemic or the 2010/11 seasonal vaccine or of natural infection.

It was discussed before the 2010/11 influenza season, whether or not this unique situation would lead to high immunity in the population and thus to a particularly mild influenza season, as it was generally assumed that the influenza A(H1N1)pdm09 virus would remain the predominant virus strain in the 2010/11 season. The proportion of a population that must be immune to reduce the mean number of secondary infections per infectious individual to less than one, is called the herd immunity threshold (HIT). The HIT indicates that a certain level of population immunity reduces the probability of infection of non-immune individuals. In viral diseases, this particular immunity threshold directly depends on the transmission potential of the infectious agent. Direct and indirect protection reduce the reproduction rate, eventually stopping or preventing an epidemic wave [3].

In addition to vaccine effectiveness and duration of protection, the degree of vaccine uptake in the population during a mass vaccination campaign is essential for the mitigation of pandemic influenza. In Germany and other countries, there was a significant media-driven debate on risks and benefits associated with adjuvanted and non-adjuvanted pandemic influenza vaccines

[4]. As a result, vaccination coverage remained low in Germany, in the general population and in risk groups [5].

In a seroprevalence study in the second largest city in Germany, we intended (i) to assess the proportion of persons with detectable antibodies against influenza A(H1N1)pdm09 and to estimate whether or not the HIT was reached, (ii) to compare antibody titres in vaccinated and previously infected persons in general and in high risk subgroups, and (iii) to obtain information on the acceptance of past pandemic vaccination campaigns and pandemic vaccines in a reference population.

Methods

Questionnaire

We performed a cross-sectional survey in Hamburg between 1 and 21 November 2010. Hamburg is the second largest city in Germany with 1.8 million inhabitants. The recruitment period was kept short to allow as many seasonal influenza vaccinations as possible to happen prior to enrolment, while excluding potential natural infections that might occur at the beginning 2010/11 influenza season. Volunteers were recruited through advertisements in the city's public transportation system. Registration for participation in the survey was possible via telephone or internet. Besides age above 18 years and ability to understand the informed consent process there were no particular in- or exclusion criteria. Basic demographic data, information on influenza-like illness, vaccination status as well as concomitant diseases or risk factors for complicated influenza were obtained using a standardised questionnaire after having obtained written consent. Each questionnaire was reviewed for completeness and consistency by a trained member of the investigation team together with the participant. Questions included whether or not the participant had received the regular seasonal influenza vaccine after April 2009 and/or the pandemic vaccine. A condition 'past influenza A(H1N1) disease' (referred to as 'natural infection' in this manuscript) was assumed if the participant reported history of influenza diagnosed by a medical doctor or treatment with neuraminidase inhibitors prescribed by a medical doctor since April 2009. Questions addressing the participants' opinion on the past vaccination campaigns as well as pandemic vaccines as such included whether or not the participant had general concerns with respect to adjuvants contained in vaccines and if yes, whether he/she would still be willing to receive adjuvanted vaccines in future pandemics.

Ethical approval was obtained from the local ethics committee of the Hamburg chamber of physicians.

Virological analysis

A serum sample was obtained from each participant, centrifuged at 2,000 g for 10 minutes, and stored at -80 °C until further processing. The samples were analysed for antibodies against influenza A(H1N1)pdm09 virus by an in-house haemagglutination inhibition (HI) test which gave clear and highly reproducible results and allowed to determine titres of 1:10 against influenza A(H1N1)pdm09 virus (sensitivity 1.0 and specificity 0.96). This in-house HI test has been described in detail elsewhere [6]. In brief, the HI-test was designed to reach a high specificity without losing relevant sensitivity and could show that there was no cross-reactivity between antibodies against seasonal influenza A/Brisbane/59/2007(H1N1) and antibodies against influenza A(H1N1)pdm09 influenza (unpublished data). The external reference serum pool obtained from the National Institute for Biological Standards and Control (NIBSC), with a defined titre after multi-laboratory testing of 1:160, however, was reproducibly equivalent to a titre of 1:80 (i.e. one two-fold dilution lower) in our test.

Statistical analysis

Statistical analyses were performed from anonymised data by members of the study team who had not been involved in the recruitment and questionnaire procedures. For calculating the HIT, we used a basic reproduction number (R_0) of 1.5 which was assumed to be the most realistic by the European Centre for Disease Prevention and Control (ECDC) [7]. This R_0 lies within a range between 1.2 and 1.7, which has been reported for Europe and the United States of America [8,9]. Using the simplified equation to calculate the HIT=$(R_0-1)/R_0$ [10], protective antibody titres would have been necessary in at least 33% (17–41%) of the population to prevent a significant influenza wave in the 2010/11 season.

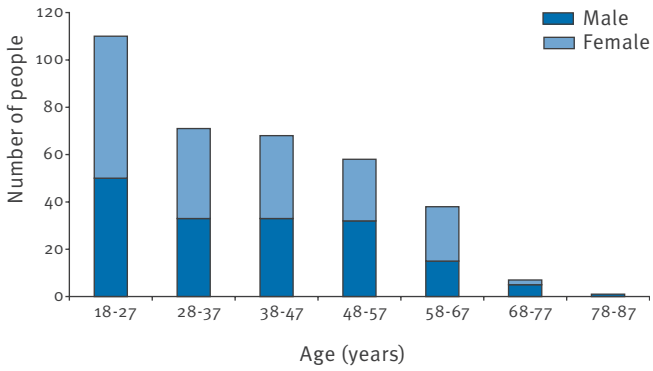
Results

Of the 353 study persons, 169 (48%) were male. Median age was 39 years (range 18–78), see Figure. Co-morbidities including diabetes, cancer, immunosuppression, and chronic liver or kidney disease were present in 52 of 353 individuals (15%).

Vaccination coverage for pandemic (49/353, 14%) and 2010/11 seasonal (40/353, 11%) influenza was very low. Ten participants (2.8%) reported to have been

Figure

Study population, stratified by age and sex, Hamburg, 1–21 November 2010 (n=353)



diagnosed with influenza (n=2) or to have been treated with neuraminidase inhibitors (n=8) since April 2009 and were, hence, classified as probable 'natural infections'. Seventy-nine individuals (22%) reported evidence for at least one of the three options, pandemic vaccination, seasonal 2010/11 vaccination or natural infection, for acquisition of specific antibodies. Of the 52 persons with co-morbidities, nine stated to have received the pandemic vaccine and a further nine stated to have received the 2010/11 seasonal influenza vaccine. Four had been vaccinated with both vaccines.

Serological analysis of the entire study population revealed influenzaA(H1N1)pdm09 antibody titres of ≥1:10 in 135 of 352 (38%) and of ≥1:40 in 61 of 352 (17%) samples (Table 1), which is below the calculated HIT of 33% for the ≥1:40 levels. A titre of ≥1:20 (which represents a titre of ≥1:40 in the external NIBSC-reference serum pool and is often deemed as protective) was reached by 97 of 352 (28%).

Proportions of subjects with antibody titres in the three subgroups (i) pandemic vaccination, (ii) seasonal influenza 2010/11 vaccination, and (iii) 'natural infection' are shown in Table 1 for titres ≥1:10 and ≥1:40. Among pandemic vaccine recipients, antibody titres ≥1:10 were detected in 39 of 49 and titres ≥1:40 in 23 of 49 people one year (range 8–14 months) after vaccination with the pandemic vaccine. Similar proportions of antibody titres of ≥1:10 and ≥1:40 were detected in those that had received the 2010/11 seasonal influenza vaccine shortly (0–10 weeks) before participating in this study: 36 of 40 vaccine recipients had antibody titres ≥1:10, 22 of 40 had titres ≥1:40. Among the 14 individuals who were sequentially immunised with both the pandemic and seasonal influenza 2010/11 vaccines, 13 exhibited titres ≥1:10 and nine titres ≥1:40. Interestingly, antibody titres ≥1:10 were detected in 71 of 272 (26%) of individuals without any history of either disease or vaccination (24/272 (9%) for titres ≥1:40), indicating asymptomatic infection in about a quarter of all participants (Table 1). Yet the proportion of individuals with positive antibody titres was significantly higher in those with a history of vaccination and/or disease than in those without for both titres, ≥1:10 (80% versus 26%, $P<0.0001$) and ≥1:40 (47% versus 9%, $P<0.0001$).

Overall, the proportion of vaccinated individuals with high antibody levels (≥1:40) decreased with age in all subgroups while such a trend was not obvious for those with antibody levels ≥1:10 (Table 1). Proportions of subjects with titres ≥1:10 and ≥1:40 were comparable in those with and without co-morbidities (≥1:10 titres: 46% versus 37%, $P=0.2$; ≥1:40 titres: 15% versus 18%, $P=0.7$).

When asked about their personal opinion about past pandemic vaccination strategies, 58% of the participants stated that mass vaccinations and respective campaigns in 2009 were not justified in retrospect. However, 63% would accept to receive pandemic vaccinations in future pandemic situations. Twenty-two percent (76/353) stated to have concerns regarding the adjuvant that was included in the pandemic vaccine. Yet 61% (46/76) of them would be open for future pandemic vaccinations despite their adjuvant-related concerns (Table 2).

Table 1

Antibody titres against influenza A(H1N1)pdm09 in the study population, stratified by age group and antibody level, after vaccination or natural infection, Hamburg, Germany, 1–21 November 2010 (n=353)

Study participants		18–39 years	40–59 years	≥60 years	All
Recipients of pandemic vaccination	Number (%)	26/193 (13)	15/125 (12)	8/35 (23)	49/353 (14)
HI-test results	Titre ≥1:10 (%)	21/26 (81)	10/15 (67)	8/8 (100)	39/49 (80)
	Titre ≥1:40 (%)	15/26 (58)	6/15 (40)	2/8 (25)	23/49 (47)
Recipients of 2010/11 seasonal vaccination	Number (%)	15/193 (8)	15/125 (12)	10/35 (29)	40/353 (11)
HI-test results	Titre ≥1:10 (%)	15/15 (100)	11/15 (73)	10/10 (100)	36/40 (90)
	Titre ≥1:40 (%)	11/15 (73)	9/15 (60)	2/10 (20)	22/40 (55)
Natural infection[a]	Number (%)	5/193 (3)	3/125 (2)	2/35 (6)	10/353 (3)
HI-test results	Titre ≥1:10 (%)	3/5 (60)	1/3 (33)	2/2 (100)	6/10 (60)
	Titre ≥1:40 (%)	2/5 (40)	0/3 (0)	0/2 (0)	2/10 (20)
No evidence for natural infection[a] or vaccination	Number (%)	154/193 (80)	99/125 (79)	20/35 (57)	273/353 (77)
HI-test results	Titre ≥1:10 (%)	53/154 (34)	15/99 (15)	3/19 (15)	71/272 (26)
	Titre ≥1:40 (%)	17/154 (11)	6/99 (6)	1/19 (1)	24/272 (9)
All	Number	193	125	35	353
HI-test results	Titre ≥1:10 (%)	86/193 (45)	31/125 (25)	18/34 (53)	135/352 (38)
	Titre ≥1:40 (%)	41/193 (21)	16/125 (13)	4/34 (12)	61/352 (17)

HI: haemagglutination inhibition.

[a] 'Natural infection' was defined as influenza disease diagnosed by a physician or treatment with neuraminidase inhibitors since April 2009.

Discussion

In the present study, the calculated HIT was not met for protective antibody levels as neither a protective titre of ≥1:40 nor a titre of ≥1:20 was reached by 33% of the study population. Approximately one third of all participants showed measurable antibody titres of ≥1:10 but this titre lies below the protective level (see below). This reflects a rather low proportion with protective or even detectable antibody levels in the study population about one and a half years after the pandemic and roughly one year after pandemic mass vaccinations in Hamburg, Germany. The true proportion of people with measurable antibody titres in the general population is likely to be even lower than the numbers presented here due to potential recruitment bias in our study population: the participants may have had a more positive view on influenza vaccinations than the general population.

When this study was conducted in November 2010 just before the beginning of the 2010/11 influenza season, antibodies against influenza A(H1N1)pdm09 could be detected in the majority of those who had received pandemic vaccination in late 2009 as well as also in those who had received seasonal 2010/11 influenza vaccination recently. While during the pandemic almost exclusively the adjuvanted influenza vaccine had been used in Germany, almost all trivalent influenza vaccines used in the 2010/11 season were non-adjuvanted vaccines. Assuming that immunity lasts longer after administration of adjuvanted vaccines (unpublished results), this might explain why the prevalence of ≥1:10 and ≥1:40 antibody titres was similar between the two groups of vaccine recipients even though the pandemic vaccine was administered one year earlier. However, in some study participants the determination of antibodies may have been performed too soon after vaccination to detect high antibody titres induced by the 2010/11 seasonal vaccine. Interestingly, a high proportion of individuals without history of vaccination or infection had detectable antibody levels. This indicates a high rate of infections with a subclinical or mild clinical course in the study population, which is in line with other seroprevalence studies [11-14]. In a recently published report by von Kries et al., who compared influenza A(H1N1)pdm09-specific antibody titres in unvaccinated children in Germany before and after the pandemic, the serologically determined incidence of pandemic influenza was as high as 25.4% in the age group of 1–4 year-olds and 28% in children aged 5–17 [6].

Overall, the proportion of vaccinated individuals with high antibody levels (≥1:40) decreased with age in all subgroups possibly reflecting declining immune response with age. However, this potentially waning immune response in the elderly was not seen at the ≥1:10 antibody level in the vaccinated subgroups possibly indicating that vaccination confers better immune response than asymptomatic infection. Vaccination coverage was also low in participants with co-morbidities generally accepted to confer a high risk for complicated influenza disease [1], and antibody levels in this group were not significantly higher compared to antibody levels in individuals without risk factors. This implies that immune protection of the sub-population with risk factors may have been insufficient before the past 2010/11 influenza season.

It is not fully established how well HI antibody titres reported in the literature correlate with protection from disease, but generally a HI-titre of ≥1:40 is regarded to indicate protection [15]. The in-house HI test used in this study was designed to be highly specific, which resulted in a two-fold lower titre when testing the external reference serum pool obtained from the NIBSC. Consequently, titres of 1:20 are equivalent to 1:40 titres obtained by less stringent HI-tests [6]. On the other hand, recently published preliminary data from the Influenza Monitoring Vaccine Effectiveness in Europe (I-MOVE) network indicate low vaccine effectiveness of the seasonal 2010/11 influenza vaccine [16,17]. Data from the United Kingdom, for example, have shown a vaccine effectiveness of the pandemic and the 2010/11 seasonal influenza vaccines against RT-PCR-confirmed influenza in the 2009/10 and 2010/11 seasons of 34% and 46%, respectively, and of 63% in those who were vaccinated in both seasons [18]. In an accompanying editorial, Puig-Barbera hypothesised the test-negative study design of the observational study or adjustments

Table 2

Vaccination history of the study participants, their concerns related to the adjuvanted pandemic vaccine and their willingness to receive pandemic vaccination in the future, Hamburg, Germany, 1–21 November 2010 (n=353)

Question	Answer	All	Male (n=169)	Female (n=184)
Have you received 2009/10 seasonal influenza vaccination?	Yes	82 (23%)	45 (27%)	37 (20%)
Are you planning 2010/11 seasonal influenza vaccination?	Yes	75 (21%)	37 (22%)	38 (21%)
Have you ever been vaccinated against seasonal influenza?	Yes	182 (52%)	91 (54%)	91 (49%)
Do you have concerns regarding adjuvants used in the pandemic vaccine?	Yes	76 (22%)	31 (18%)	45 (24%)
Would you agree to receive pandemic vaccination in the future?	Yes (all)	206 (58%)	93 (55%)	113 (61%)
	Yes (those concerned about adjuvants)	46/76 (61%)	20/31 (65%)	26/44 (59%)

for some variables in the analyses might have led to an underestimation of the vaccine effectiveness [19]. When applying an arbitrary cut-off titre of ≥1:40, our data are in line with the observed low vaccine effectiveness. Nevertheless, influenza A(H1N1)pdm09 antibodies were significantly more often detected in those who had received pandemic or seasonal vaccination than in those that had not. Interestingly, in our study the small number of participants who had been vaccinated with both the pandemic and the seasonal vaccine exhibited highest antibody titres of ≥1:10 in 93% and ≥1:40 in 64%, possibly suggesting a prime-boost effect.

Vaccination coverage was very low in our study population. In Sweden, vaccination coverage with the same pandemic vaccine was higher than 60% in the general population and higher than 95% in healthcare workers [20]. According to a representative telephone survey, vaccination coverage in Hamburg after the influenza A(H1N1)pdm09 vaccination campaign was as low as 8.4% (95% confidence interval: 4.9–14.0) [12]. The fact that the vaccination coverage in our study population was higher than in the telephone survey suggests our study sample may have suffered from a recruitment bias as discussed above. Nevertheless, as other studies also found low vaccination coverage for pandemic influenza in Hamburg and other German regions [4], it is reasonable to assume that our data on vaccine-induced immunity can be generalised for the entire German population.

In Germany, there was a vivid media-driven debate on risks and benefits associated with the mass vaccination campaigns and the adjuvant containing squalene used in pandemic vaccines [4,5]. A high proportion of participants stated to have had reservations regarding the adjuvant and the usefulness of the mass vaccination campaigns. Nevertheless, concerns related to the adjuvant did not influence the participants' willingness to receive pandemic vaccination in the future. This could be interpreted to indicate that the low vaccination coverage in our study population (and possibly in all of Germany) was related to personal distrust of mass vaccination campaigns and adjuvants in general rather than of the effectiveness of the pandemic vaccine. This information could be important for planning health protection measures in future pandemics in that it may be important to provide more detailed information on the vaccine and to justify both active and non-active ingredients, i.e. the form of the active ingredient (whole virion versus split/subunit vaccines), the adjuvant as well as non-active excipients.

The present study has several limitations. In addition to the recruitment bias, the way this study was conducted includes the risk of recall bias in that some participants may not exactly remember the type and date of vaccinations received one and a half years before the study or may be unable to provide reliable information on influenza-like illness. We do not think, however, that this had a significant impact on our major findings as the study participants are likely to remember precisely whether or not they have received pandemic vaccination because of the particular circumstances around the pandemic vaccinations (such as information, specific locations or waiting lists), while seasonal 2010/11 vaccination has only been available for a few weeks prior to this study. Furthermore, information bias may have been introduced by imprecise definitions; for example, the definition of 'natural infection' may not reflect true influenza infection due to the unspecific disease symptoms and the fact that influenza disease is usually not confirmed by laboratory testing. We are also aware of the fact that the exclusion of children and adolescents limit our findings because these groups are at high risk for influenza A(H1N1)pdm09 infection and important for its transmission [6]. While the sex distribution in the study population resembled the general population in Hamburg and Germany overall, the age distribution differed in that a comparatively high number was recruited in the younger age groups while the elderly population was underrepresented [21]. Nevertheless, the population recruited for this survey through advertisements in the public transport system represented a relevant population with respect to influenza transmission that was representative for the city of Hamburg as well as other regions in Germany. Finally, a few persons with positive antibody titres may have been missed by using the in-house HI test alone without adding the sensitivity of a second test (i.e. a microneutralisation test), but we think that a slight increase in sensitivity would have had a negligible influence on the results [15].

In conclusion, the HIT for influenza A(H1N1)pdm09 had not been reached in Hamburg, Germany by November 2010, leaving the majority of the population susceptible to the infection and its potential complications, although everyone had the chance to acquire specific immunity either during the mass vaccination campaign in 2009 (which targeted the total population in Germany), during routine seasonal influenza vaccination in 2010 (targeting specific risk groups) or by natural infection. Our data confirm the view that vaccination and potentially re-vaccination with influenza A(H1N1) pdm09-containing vaccines in 2009/10 and 2010/11 induced measurable specific antibodies. It is important to increase the proportion of immune persons in a population above the HIT with effective and fast acting vaccines especially in a pandemic situation characterised by a general lack of pre-existing specific immunity. While protective antibody titres against influenza viruses have been well defined for the purpose of assessing immunogenicity of vaccines, their correlation with clinical protection especially with regard to long-term protection is less clear. Therefore it seems important to validate the currently used serological correlates of protection against influenza viruses [15] and to continue the monitoring of breakthrough infections in observational studies during future influenza seasons as it has been done by I-MOVE.

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