Altered Response to A(H1N1)pnd09 Vaccination in Pregnant Women: A Single Blinded Randomized Controlled Trial

Anne Louise Bischoff1, Nilofar Vahman Følsgaard1, Charlotte Giwercman Carson1, Jakob Stokholm1, Louise Pedersen1, Maria Holmberg1, Amalie Bisgaard1, Sune Birch1, Theodore F. Tsai2, Hans Bisgaard1*

1 Copenhagen Prospective Studies on Asthma in Childhood (COPSAC), Health Sciences, University of Copenhagen, Copenhagen University Hospital, Gentofte, Denmark, 2 Novartis Vaccines, Cambridge, Massachusetts, United States of America

Abstract

**Background:** Pregnant women were suspected to be at particular risk when H1N1pnd09 influenza became pandemic in 2009. Our primary objective was to compare the immune responses conferred by MF59®-adjuvanted vaccine (Focetria®) in H1N1pnd09-naive pregnant and non-pregnant women. The secondary aims were to compare influences of dose and adjuvant on the immune response.

**Methods:** The study was nested in the Copenhagen Prospective Studies on Asthma in Childhood (COPSAC2010) pregnancy cohort in 2009-2010 and conducted as a single-blinded block-randomised [1:1:1] controlled clinical trial in pregnant women after gestational week 20: (1) 7.5 μg H1N1pnd09 antigen with MF59-adjuvant (Pa7.5 μg); (2) 3.75 μg antigen half MF59-adjuvanted (Pa3.75 μg); (3) 15 μg antigen unadjuvanted (P15 μg); and in non-pregnant women receiving (4) 7.5 μg antigen full adjuvanted (NPa7.5 μg). Blood samples were collected at baseline, 3 weeks, 3 and 10 months after vaccination, adverse events were recorded prospectively.

**Results:** 58 pregnant women were allocated to Pa7.5 μg and 149 non-pregnant women were recruited to NPa7.5 μg. The sero-conversion rate was significantly increased in non-pregnant (NPa7.5 μg) compared with pregnant (Pa7.5 μg) women (OR = 2.48 [1.03–5.95], p = 0.04) and geometric mean titers trended towards being higher, but this difference was not statistically significant (ratio 1.27 [0.85–1.93], p = 0.23). The significant titer increase rate showed no difference between pregnant (Pa7.5 μg) and non-pregnant (NPa7.5 μg) groups (OR = 0.49 [0.13–1.85], p = 0.29).

**Conclusion:** Our study suggests the immune response to the 7.5 μg MF59-adjuvanted Focetria® H1N1pnd09 vaccine in pregnant women may be diminished compared with non-pregnant women.

**Trial Registration:** ClinicalTrials.gov NCT01012557

Citation: Bischoff AL, Følsgaard NV, Carson CG, Stokholm J, Pedersen L, et al. (2013) Altered Response to A(H1N1)pnd09 Vaccination in Pregnant Women: A Single Blinded Randomized Controlled Trial. PLoS ONE 8(4): e56700. doi:10.1371/journal.pone.0056700

Editor: Li-Min Huang, National Taiwan University Hospital, Taiwan

Received September 10, 2012; Accepted January 14, 2013; Published April 18, 2013

Copyright: © 2013 Bischoff et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: The nested vaccine study is funded by Novartis Vaccines and Diagnostics GmbH, Marburg, Germany, who provided vaccines and funding as an unrestricted institutional grant. The main Copenhagen Prospective Study on Asthma in Childhood birth cohort study is funded by private and public research funds (see www.copsac.com). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: TFT is employed by Novartis Vaccines. The nested vaccine study is funded by Novartis Vaccines and Diagnostics GmbH, Marburg, Germany, who provided vaccines and funding as an unrestricted institutional grant. The main Copenhagen Prospective Study on Asthma in Childhood birth cohort study is funded by private and public research funds (see www.copsac.com). There are no further patents, products in development or marketed products to declare. This does not alter the authors’ adherence to all the PLOS ONE policies on sharing data and materials, as detailed online in the guide for authors.

* E-mail: bisgaard@copsac.com

Introduction

Pregnant women experience increased influenza related morbidity and mortality during seasonal influenza epidemics,[1–3], and even graver outcomes during influenza pandemics [4,3]. The WHO and many countries therefore prioritized pregnant women among those to receive the first available doses of H1N1pnd09 vaccine [6,7]. Indeed, influenza hospital admission and mortality rates were higher for pregnant women than in the general population during the 2009 pandemic [8–10]; in addition, infected pregnant women experienced increased rates of stillbirth, perinatal and neonatal mortality than their non-infected counterparts [11]. The pathophysiology of increased influenza-related morbidity during pregnancy is not fully defined but an altered immune response to infection associated with immunological adaptations to pregnancy itself may contribute [12]. The immune response of pregnant women to influenza vaccine has not been well studied, as vaccination during pregnancy has not been recommended until recently [2,3,13,14]. Although it has been widely held that responses to influenza vaccine in pregnant women and non-pregnant women are indistinguishable [15,16], a recent study
reported that antibody responses to seasonal influenza vaccination were lower in pregnant than in non-pregnant women [17]. Moreover, the reduced immune response of pregnant women to live-attenuated yellow fever vaccine suggests that the immune response of pregnant women to other antigens also may be impaired [18]. To our knowledge, no studies comparing immune responses to the H1N1pnd09 vaccine in pregnant and non-pregnant women have been reported.

We had the unique opportunity to study the H1N1pnd09 vaccination of pregnant and non-pregnant women in our unselected, prospective, clinical pregnancy-cohort: the Copenhagen Prospective Study on Asthma in Childhood 2010 (COPSAC2010) recruited between Q1-2009 and Q4-2010. The timing of this enrolment and the pandemic provided for an “experiment of nature” in our population of subjects of whom half had completed pregnancy before the pandemic and the other half were pregnant while H1N1pnd09 virus was prevalent in the community.

We conducted a randomized controlled clinical trial primarily to compare immunogenicity of the H1N1pnd09 vaccine in pregnant versus non-pregnant women; secondarily to study dose-related immune responses and adverse events to MF59-adjuvanted versus non-adjuvanted vaccine in pregnant recipients.

Materials and Methods

This study is reported in accordance with the CONSORT guidelines [19]. The protocol (Protocol S1) for this trial and supporting CONSORT checklist (Checklist S1) are available as supporting information.

Study design

This study was nested in the novel COPSAC2010 cohort: an ongoing, unselected, prospective clinical pregnancy cohort study of 743 women recruited in Zealand, Denmark, during 2009-2010. The recruitment was previously described in detail [20].

The women participating in the COPSAC2010 cohort were invited to be enrolled in this phase IV randomized, participant-blinded study in 2009–2010. The pregnant women were recruited from gestational week 20 and women continuing in the birth cohort with their children were recruited up to 8 months after birth. Key exclusion criteria were: chronic endocrinological, nephrological or cardiac diseases; severe asthma; history of anaphylaxis or other serious vaccine reactions; or hypersensitivity affecting mother or child were recorded. Any reaction that interfered with daily activity; severe (prevented daily activity); and severe (prevented daily activity). At two further clinical visits, after three and 10 months, unsolicited events were graded as: none; mild (did not interfere with daily activity); moderate (interfered with daily activity); and severe (prevented daily activity). At two further clinical visits, after three and 10 months, unsolicited events affecting mother or child were recorded. Any reaction that resulted in hospitalization or was life-threatening was considered as a serious adverse event.

Adverse events

The women visited the research clinic after three weeks for a structured clinical interview performed by the research doctors interviewing for specific local reactions (pain, erythema, swelling, and bruising), systemic symptoms (chills, malaise, headache, myalgia, nausea, and vomiting), fever, and use of analgesics. Symptoms and reactions were graded as: none; mild (did not interfere with daily activity); moderate (interfered with daily activity); and severe (prevented daily activity). At two further clinical visits, after three and 10 months, unsolicited events affecting mother or child were recorded. Any reaction that resulted in hospitalization or was life-threatening was considered as a serious adverse event.

Serology

Serum was sampled prior to vaccination (baseline), 3 weeks, 5 months, and 10 months after vaccination. Serum plasma levels of antibodies were determined in twofold dilutions in a conventional hemagglutination-inhibition (HI) assay [23], using turkey red blood cells [24]. Four hemagglutination units of a live viral preparation of A/GA/07/2009 (the strain contained in the pandemic vaccine), were used as the antigen. The coded samples were analyzed at Novartis Vaccines and Diagnostics, Marburg, Germany.

Sero-conversion was defined as a change from a pre-vaccination HI titer <10 to a post-vaccination HI titer ≥40; significant increase as fourfold or higher post-vaccination titer from a pre-vaccination HI titer ≥10, and sero-protection as the proportion of subjects achieving an HI titer of 40 or greater. See Table 1 for serology definitions.

Table 1. Definitions.

| Sero-protection: | HI titer ≥40 |
|------------------|-------------|
| Sero-conversion: | Pre-vaccination: HI titer ≥40 |
|                  | Post-vaccination: HI titer ≥40 |
| Significant increase: | Pre-vaccination: HI titer ≥10 |
|                  | Post-vaccination: HI titer ≥4 x pre-vaccination titer |

doi:10.1371/journal.pone.0056700.t001

The vaccines were portioned, in identical syringes, into two different individual dosages. One for the 7.5 µg full MF59-adjuvanted and 3.75 µg half MF59-adjuvanted dosages and one for the 15 µg unadjuvanted dose. The vaccine was administered as an intramuscular injection into the deltoid muscle. The women were observed for 30 minutes after the injection.

Ethics Statement

The study was conducted in accordance with the guiding principles of the Declaration of Helsinki and approved by the Ethics Committee of Copenhagen (H-B-2008-093), the Danish Medicines Agency (EudraCT 2009-016577), and the Danish Data Protection Agency (2009-41-0031). The validity of the data was ensured by complying with Good Clinical Practice guidelines and quality-control procedures. (ClinicalTrials.gov number, NCT01012557). All participants gave their written informed consent prior to enrollment.
The HI antibody response-criteria for success in young adults as defined by the EMEA criteria [25] of successful response in young adults are: (1) 40% of subjects with sero-conversion or significant titer increase, (2) 70% of subjects achieving sero-protection; or a (3) Geometric Mean Ratio (GMR) between pre- and post-vaccination titers of >2.5.

Statistics

The study power estimate was based on a minimal detectable Geometric Mean Titer (GMT) ratio of 1.5 between the Pa7.5 μg and the NPa7.5 μg groups, a coefficient of variation (CV) of 1.0, a ratio of 1:3 between number of pregnant and number of non-pregnant women, a power of 80%, and an two-sided alpha-level of 5%. This required us to recruit a total number of 180 women in the Pa7.5 μg and the NPa7.5 μg groups.

GMT were computed from the log10-transformed mean. GMT in the vaccination groups are compared in levels and trends over time using general estimating equations (GEE) analysis (repeated-measures analysis) with an independent working correlation structure. The motivation for using GEE analysis is to take advantage of the full data in order to report one overall conclusion on the difference in sero-conversion rates between the groups over time. Differences in GMT between groups are reported as ratios (R). Confidence intervals are reported in squared brackets. GMT at 3 weeks, 3 and 10 months are adjusted for baseline GMT, using baseline GMT as a covariate. Analysis on the Pa7.5 μg, Pa3.75 μg and P15 μg groups were adjusted for gestational age at time of vaccination.

GMR are the ratio between baseline titer and titer at any time point.

Percentage of women sero-converted is calculated as the percentage of women with a pre-vaccination HI titer >10 who converted to a post-vaccination HI titer ≥40. Percentage of women with significant titer increase is calculated as the percentage of women with a pre-vaccination HI titer >10 who had a fourfold or higher post-vaccination titer. Percentage of women with sero-conversion or significant increase is calculated as percentage of the total number of women in the vaccination group.

Sero-protection, sero-conversion and significant titer increase in the vaccination groups are compared in levels and trends over time using general estimating equations (GEE) analysis (repeated-measures analysis) with an independent working correlation structure. Differences in sero-conversion and significant titer increases between groups are reported as longitudinal odds ratios (OR). Confidence intervals are reported in squared brackets. Due to large scatter in gestational age at time of vaccination in the pregnant women, analyses on the Pa7.5 μg, Pa3.75 μg and P15 μg groups were adjusted for gestational age at time of vaccination.
Table 2. Baseline characteristic of the study subjects according to vaccine group.

|                      | Pa7.5μg MF59-adjuvanted | Pa3.75μg MF59-adjuvanted | P15μg Not-adjuvanted | NPa7.5μg MF59-adjuvanted | P  |
|----------------------|-------------------------|--------------------------|----------------------|-------------------------|----|
| N                    | 58                      | 28                       | 61                   | 149                     |    |
| Age – year Median (Range) | 32(19–40)             | 34(26–40)                | 31(20–41)            | 32(23–49)               | 0.12* |
| Gestational age at vaccination – weeks Median (Range) | 25+0 (21+4 – 40+1) | 29+0 (21+1 – 38+6) | 24+6 (20+0 – 37+0) | *                        |
| Time from birth to vaccination – days Median (Range)           |                          |                          |                      | 127 (6–244)            |
| Income (pr. year) % (n)                                      |                          |                          |                      |                          |
| <400,000 Dkk          | 9(5)                    | 4(1)                     | 22(13)               | 13(19)                  | 0.08† |
| 400,000–1,000,000 Dkk | 82(45)                  | 96(27)                   | 73(43)               | 84(125)                 |    |
| >1,000,000 Dkk        | 9(5)                    | 0                        | 5(3)                 | 3(5)                    |    |
| Previous births % (n)                                      |                          |                          |                      |                          |
| Yes                  | 48(28)                  | 68(19)                   | 64(39)               | 55(82)                  | 0.21† |
| No                   | 51(30)                  | 32(9)                    | 36(22)               | 45(67)                  |    |
| Living with partner % (n)                                  |                          |                          |                      |                          |
| Yes                  | 100(58)                 | 100(28)                  | 98(60)               | 97(144)                 | 0.69 ‡ |
| No                   | 0                       | 0                        | 2(1)                 | 3(5)                    |    |

*ANOVA.
†Fisher’s exact test.
*The gestational age at time of vaccination is different in the Pa3.75 μg group compared with the Pa7.5 μg group (P = 0.01). There is no significant difference between Pa7.5 μg and P15 μg (P = 0.82). Wilcoxon Rank Sum Test (t approximation).

doi:10.1371/journal.pone.0056700.t002

Figure 2. Evolution of geometric mean titers in the four vaccine groups over time. The trends over time for geometric mean titers in the vaccine groups were not significantly different using general estimating equations.
doi:10.1371/journal.pone.0056700.g002
Local and systemic post-vaccination reactions are reported as percentages based on number of women and severity. We used Fisher’s exact test to compare vaccine groups. Exact (Clopper-Pearson) confidence intervals are reported for all proportional endpoints in squared brackets.

A p-value $\leq 0.05$ was considered as significant.

Analyses were done using SAS version 9.2 (SAS Institute, Cary, NC).

### Results

#### Baseline characteristics

296 women were included in this study from November 2009 to August 2010 of which 149 were non-pregnant (NPa7.5 μg). The 147 pregnant women were randomly assigned to the three groups Pa7.5 μg (58 women), Pa3.75 μg (28 women), and P15 μg (61 women). The Pa3.75 μg group was closed after four months due to a lower-than-expected recruitment rate to the study. There was a follow-up of 87% of the women 10 months after vaccination and the study was closed July 2011. Flow of subjects into the study-

---

### Table 3. Antibody responses according to vaccine groups.

| Vaccine group | Pa7.5μg | Pa3.75μg | P15μg | NPa7.5μg |
|---------------|---------|----------|-------|----------|
| **Baseline**  |         |          |       |          |
| N             | 58      | 28       | 61    | 149      |
| GMT [95% CI]  | 10[8–13]| 11[7–17]| 9[7–12]| 12[10–14]|
| Sero-protection [95% CI] | 17[9–29]| 17[6–37]| 13[6–24]| 13[8–19]|
| **3 Weeks**   |         |          |       |          |
| N             | 56      | 28       | 59    | 148      |
| GMT [95% CI]  | 345[244–487]| 206[126–335]| 202[145–283]| 465[374–575]|
| GMR [95% CI]  | 33.2[22.5–49.2]| 18.8[10.8–32.8]| 21.7[14.8–31.8]| 40.5[31.8–51.5]|
| Sero-protection [95% CI] | 96[88–100]| 89[72–98]| 88[77–95]| 99[95–100]|
| Sero-conversion [95% CI] | 95[82–99]| 86[57–98]| 83[67–93]| 98[91–100]|
| Significant increase [95% CI] | 95[74–100]| 93[66–100]| 89[67–99]| 86[76–94]|
| Sero-conversion or Significant increase [95% CI] | 95[85–99]| 89[72–98]| 85[73–93]| 93[87–96]|
| **3 Months**  |         |          |       |          |
| N             | 49      | 27       | 58    | 147      |
| GMT [95% CI]  | 139[93–208]| 87[51–150]| 111[77–161]| 207[164–261]|
| GMR [95% CI]  | 12.5[8.1–19.3]| 8.0[4.4–14.4]| 11.5[7.7–17.1]| 18.0[14.0–23.1]|
| Sero-protection [95% CI] | 82[68–91]| 74[54–89]| 76[63–86]| 94[89–97]|
| Sero-conversion [95% CI] | 70[51–85]| 64[35–87]| 74[58–87]| 91[83–96]|
| Significant increase [95% CI] | 84[60–97]| 69[39–91]| 63[38–84]| 79[67–88]|
| Sero-conversion or Significant increase [95% CI] | 76[61–87]| 67[46–83]| 71[57–82]| 86[79–91]|
| **10 Months** |         |          |       |          |
| N             | 47      | 24       | 50    | 135      |
| GMT [95% CI]  | 75[48–118]| 63[34–118]| 85[55–130]| 78[60–101]|
| GMR [95% CI]  | 6.8[4.2–10.8]| 5.5[2.9–10.6]| 8.4[5.3–13.2]| 6.8[5.1–8.9]|
| Sero-protection [95% CI] | 70[55–83]| 67[45–84]| 74[60–85]| 75[66–82]|
| Sero-conversion [95% CI] | 59[39–76]| 55[23–83]| 70[51–84]| 69[57–79]|
| Significant increase [95% CI] | 67[41–87]| 54[25–81]| 53[28–77]| 59[46–71]|
| Sero-conversion or Significant increase [95% CI] | 62[46–75]| 54[33–74]| 64[49–77]| 64[56–72]|

P: Pregnant.
NP: Non-pregnant.
GMT: Geometric mean titer.
GMR: Geometric mean ratio.
CI: Confidence interval.
Sero-protection: Titer $\geq 40$.
Sero-conversion: Pre titer $<10$, Post titer $\geq 40$.
Significant increase: Pre titer $\geq 10$, Post titer 4 fold Pre titer.
Sero-conversion or significant increase: Percentage of total number of women in each vaccine group.

1 At day 0 GMT were the same in all groups (ANOVA, p = 0.51).
2 The number of women with sero-protection did not differ in any of the groups compared over time (GEE).
3 GMT at 3 weeks, 3 months and 10 months were adjusted for baseline titer.

doi:10.1371/journal.pone.0056700.t003
analysis is illustrated in the Figure 1, and the baseline characteristics of the study groups in Table 2.

**Immunogenicity**

We saw an overall successful sero-protective response from the H1N1pdm09 vaccination in more than 91% of pregnant and 99% of non-pregnant women as defined by one of the EMEA criteria [25]. The detailed immune responses are presented in Table 3.

The CV for GMT was 1.8. Subjects with baseline titers were found in all groups. Therefore, GMT at 3 weeks, 3 months and 10 months were adjusted for baseline titer (Figure 2 and Table 3).

**Time-trend.** The interactions with time of the vaccine groups were not significant; i.e. the trends over time for the number of women sero-converted, and for the women with significant titer increase were not significantly different when comparing pregnant (Pa7.5 μg) versus non-pregnant (NPa7.5 μg) women, comparing Pa3.75μg group versus Pa7.5 μg group and comparing P15 μg group versus P7.5 μg group (Figure 2-3).

**Pregnant versus non-pregnant women.** GMT numerical values (Table 3) suggested a lower response in pregnant women (Pa7.5 μg) compared with non-pregnant women (NPa7.5 μg) but this was not significant (R of non-pregnant vs pregnant women: 1.27 [0.83–1.93], p = 0.23) (Figure 2, Table 3). The number of non-pregnant women (NPa7.5μg) who sero-converted was 2.48-fold higher than among pregnant (Pa7.5 μg) women (OR = 2.48 [1.03–5.95], p = 0.04) (Figure 3, Table 3). The number of women with significant titer increase from baseline was not significantly different (OR = 0.49 [0.13–1.85], p = 0.29) (Table 3).

**Dose-related response in pregnant woman.** There was no significant difference between the Pa3.75μg group and the Pa7.5 μg group with respect to GMT (R = 0.63 [0.33-1.24], p = 0.18) (Figure 2, Table 3; sero-conversion (OR = 0.65 [0.18-2.39], p = 0.52) (Figure 3, Table 3) or the number of women with significant titer increase in the two groups (OR = 0.41 [0.10–1.77], p = 0.23) (Table 3). Importantly, the confidence limits are very wide corresponding to the low number of participants in the P3.75 μg group.

**Response to non-adjuvant, double-dose vaccine in pregnant women.** There was no significant difference between the P15μg group and the Pa7.5 μg group with respect to GMT (R = 0.83 [0.48–1.42], p = 0.49) (Figure 2, Table 3; sero-conversion (OR = 1.11 [0.42–2.91], p = 0.83) (Figure 3, Table 3) or the number of women with significant titer increase in the two groups (OR = 0.33 [0.07–1.55], p = 0.16) (Table 3).

**Safety analysis**

Local and systemic reactions in the first 3 weeks following vaccination are shown in Table 4 and 5. Overall, 72% of women reported adverse reactions, 23% reported no adverse reactions,
and 4% had missing information on the adverse reactions. 64% experienced local reactions after vaccination. The women in the P15 μg group experienced significantly fewer local reactions than in the Pa7.5 μg and NPa7.5 μg groups with respect to pain (P<0.000001), erythema (P = 0.03), and swelling (P = 0.008) (Table 4). 26% of the women reported systemic reactions, with malaise as the most common symptom in 20% of the women. There were no significant differences between reported systemic reactions in the four study groups (Table 5). There were no serious adverse events in this study.

Discussion

Principal Findings

The sero-conversion OR was 2.48-fold in non-pregnant women (NPa7.5 μg) compared with pregnant women (Pa7.5 μg) after receiving the same standard H1N1pnd09 vaccine. Likewise GMT was nominally higher in the non-pregnant than in the pregnant women.

As young adults were at high risk for illness during the pandemic, it was of interest that even after 10 months, 70–74% of pregnant (Pa7.5 μg and P15 μg) and 75% non-pregnant women (NPa7.5 μg) were protected against H1N1 according to the EMEA criteria with a HI titer of 40 or greater.

Women receiving the non-adjuvanted vaccine had significantly fewer local reactions but similar rates of systemic reactions as women receiving the adjuvanted vaccine. There were no reports of serious adverse events in any of the groups.

Strengths and Limitations of the Study

It is a major strength to this study that it is nested in the ongoing well established COPSAC2010 cohort study, which ensured a high follow-up rate and close observations in a clinical research centre.

The non-pregnant subjects were post-partum women drawn from the parent study and had delivered within 8 months prior to vaccination. As immune changes associated with pregnancy may be carried over transiently to the post-partum period, this would have had the effect of minimizing differences between pregnant and non-pregnant subjects, meaning that our observations were conservative.

It is a limitation of this study, that the recruitment was hampered by two main factors; primarily the Danish Health Authorities were ambiguous in their recommendations for vaccination of pregnant women changing their recommendations during the pandemic. This ambivalence caused the women to doubt the necessity and safety of the offered vaccination. Secondly the clinical symptoms of the pandemic proved less serious than expected. Therefore, failing to recruit at the scheduled rate, we chose to close the Pa3.75 μg treatment arm halfway into the recruitment period. The low number of participants in this study arm limits the power of the conclusion on this particular treatment.

As a consequence of lower than expected recruitment the power to detect differences between study arms was lower than planned.

The response to the unadjuvanted vaccine containing the usual 15 μg of antigen in the pregnant women can only be compared to the adjuvanted vaccine containing 7.5 μg of antigen in the non-

Figure 3. Evolution of sero-conversion rate (as percentage) in women in the four vaccine groups over time. The trends over time for the number of women sero-converted were not significantly different. The number of non-pregnant women (NPa7.5 μg) who sero-converted was 2.48-fold higher than among pregnant (Pa7.5 μg) women using general estimating equations.

doi:10.1371/journal.pone.0056700.g003
pregnant women, since this study did not include a non-pregnant 15 μg dose unadjuvanted group. Hence we could not report on the adequacy of the usual unadjuvanted 15 μg antigen dose in pregnancy. It is a limitation of the study that it was not observer blind; but the extensive interviews for adverse events were part of the comprehensive clinic interviews in the main study with a focus on asthma, eczema and allergy and were integrated within the framework of the on-going COPSAC2010 study.

The serum plasma levels of antibodies were determined by only the HI assay. Using a second method e.g. the microneutralization assay [26], to determine the serum plasma levels of antibodies and comparing these values would have strengthened our results.

Finally, it is a limitation to our study (though inevitable), that the baseline GMT was raised in certain individuals in all four groups, indicating that some individuals in the population were already protected against the novel influenza H1N1pdm09. This could be due to the cross-reactivity from other influenza viruses [27,28]. An earlier study on the Focetria vaccine showed that the presence of a high baseline titer did not have any influence on the rate of seroconversion or titer increase [29].

**Interpretation**

The increased risk for severe influenza in pregnant women was demonstrated again in the 2009 pandemic, underscoring the importance of protecting this vulnerable group by vaccination and with antiviral therapy [8–10]. Therefore the public health and medical imperative to protect pregnant women from influenza is clear and routine vaccination is increasingly recommended.

Vaccinating pregnant women against influenza may have a secondary benefit by passively protecting the parturient woman’s infant,[30–33], and higher antibody titers achieved in pregnancy may increase the efficacy or duration of that transmitted immunity.

**Table 5.** Solicited systemic reactions the first 3 weeks following vaccination.

|                  | Pa7.5 μg MF59-adjuvanted | Pa3.75 μg MF59-adjuvanted | P15 μg Not-adjuvanted | NPa7.5 μg MF59-adjuvanted |
|------------------|--------------------------|---------------------------|-----------------------|--------------------------|
| N                | 58                       | 28                        | 61                    | 149                      |
| Missing data     | 5(3)                     | 7(2)                      | 8(5)                  | 2(3)                     |

| Systemic reaction | Percentage [95% confidence interval] |
|-------------------|--------------------------------------|
| **Chills**        |                                      |
| None              | 95[85–99]                            |
| Mild              | 5[1–15]                              |
| Moderate          | 0[0–6]                               |
| Severe            | 0[0–6]                               |
| **Malaise**       |                                      |
| None              | 78[65–88]                            |
| Mild              | 16[8–29]                             |
| Moderate          | 5[1–15]                              |
| Severe            | 0[0–6]                               |
| **Headache**      |                                      |
| None              | 89[78–96]                            |
| Mild              | 9[3–20]                              |
| Moderate          | 2[0–10]                              |
| Severe            | 0[0–6]                               |
| **Myalgia**       |                                      |
| None              | 91[80–97]                            |
| Mild              | 9[3–20]                              |
| Moderate          | 0[0–6]                               |
| Severe            | 0[0–6]                               |
| **Nausea**        |                                      |
| None              | 100[94–100]                          |
| Mild              | 0[0–6]                               |
| Moderate          | 0[0–6]                               |
| Severe            | 0[0–6]                               |
| **Vomiting**      |                                      |
| None              | 100[94–100]                          |
| Mild              | 0[0–6]                               |
| Moderate          | 0[0–6]                               |
| Severe            | 0[0–6]                               |
| **Fever**         |                                      |
| None              | 98[90–100]                           |
| >38°C             | 2[0–10]                              |
| **Analgesics**    |                                      |
| None              | 95[85–99]                            |
| Yes               | 5[1–15]                              |

Mild: Not interfering with daily activity.
Moderate: Interfering with daily activity.
Severe: Preventing in engaging in daily activity.

doi:10.1371/journal.pone.0056700.t005
Infants under 6 months of age have high rates of serious influenza-related disease leading to hospitalizations while no influenza vaccine is licensed for children below 6 months old; therefore, routine vaccination of pregnant women has been proposed as a means to protect them indirectly [2,33–38]. An additional unexpected benefit of vaccinating pregnant women during the pandemic was an improved outcome of pregnancy, including a ~30% reduction in premature births, and reduced risks for fetal death and delivering small for gestational age neonates [39,40].

The increased risk for severe influenza in pregnant women is not fully understood. Physiological changes associated with pregnancy play a role in increased severity of influenza. Immunity against secondary infections is also potentially important. At least one third of the deaths due to the pandemic were complicated by secondary bacterial infection [41]. While overall IgG levels normally decline in pregnancy, that depression has not, to our knowledge, been shown to lead to a reduction of influenza HI antibody titers or to contribute to the increased severity of influenza in pregnancy [42]. While antibodies are the principal means of protection against acquiring influenza, cytotoxic T cells do contribute to controlling the severity of infection by clearing viral infected cells. The immune response to vaccination in pregnant and non-pregnant women has been presumed to be similar, but supporting evidence is scarce [15,16]. Immunological adaptations to pregnancy lead principally to a relative reduction of Th1 responses while protection against influenza in young adults is provided by antibodies (Th2) – conventionally measured by HI [43–45]. We showed that responses to vaccination in pregnant women may be slightly compromised compared with non-pregnant women, as demonstrated by a 2.48-lower rate of seroconversion and lower GMT responses, compared with non-pregnant women receiving the identical MF59-adjuvanted vaccine containing 7.5 μg of HA. This observation is consistent with a report that pregnant women responded less well to unadjuvanted seasonal trivalent influenza vaccine, compared to non-pregnant control women [17]. It is unclear if this difference has any clinical consequence. Serological correlates of protection, established in human challenge studies and in field trials, have shown that, in young adults, an HI titer of ~40 correlates with protection against acquiring illness [46,47]. Given the potential for severe outcomes of pandemic influenza in pregnant women, [12,45,48], our data suggest the need for further comparisons of the responses in pregnant women to influenza viral strains with a pandemic potential with a view to confirm the effectiveness of the unadjuvanted 15 μg dose and potential benefits of higher doses to obtain sufficient protection of the pregnant woman herself and her infant [33,49].

The response of pregnant women to unadjuvanted vaccine (containing 15 μg of HA) seemed adequate, though we observed numerically higher GMTs in response to adjuvanted vaccine containing one half the antigen, and equal responses to adjuvanted vaccine containing one fourth the antigen (3.75 μg). This degree of antigen-sparing from adjuvants has been observed consistently in previous studies in children, young and older adults [21,22,28], and has global public health implications because of the inadequate world supply of influenza vaccine [50]. Also consistent with previous experience, the adjuvanted vaccine was more locally reactogenic but did not increase systemic adverse reactions, including fever [29,51]. This is an important feature for a vaccine to be administered in pregnancy, as fever may be teratogenic [52–54]. We observed no serious adverse events related to any of the vaccines.

Conclusion

We find a significantly lower number of pregnant women who sero-converted than among non-pregnant women. Our study suggests a trend towards a reduced immune response to the 7.5 μg MF59 adjuvanted Focetria H1N1pdm09 vaccine in pregnant compared with non-pregnant women. The adjuvanted vaccine was significantly more locally reactogenic but provided a similar and possibly higher immune response compared to non-adjuvanted, 15 μg H1N1pdm09 vaccine in pregnant women.

Supporting Information

Checklist S1 CONSORT Checklist. (DOC)
Protocol S1 Trial Protocol. (DOC)

Acknowledgments

We gratefully express our gratitude to the children and families of the COPSAC2010 cohort study for all their support and commitment. We acknowledge and appreciate the unique efforts of the COPSAC research team. Novartis Vaccines and Diagnostics, Marburg, Germany are appreciated for technical support.

Data sharing

The protocol, the technical appendix, statistical code, and dataset are available from the corresponding author at: bisgaard@copsac.com.

Author Contributions

Conceived and designed the experiments: HB ALB NVF. Performed the experiments: ALB NVF. Analyzed the data: ALB SB. Contributed reagents/materials/analysis tools: HB TFF. Wrote the paper: ALB NVF. The protocol, the technical appendix, statistical code, and dataset are available from the corresponding author at: bisgaard@copsac.com.

References

1. Neuzil KM, Reed GW, Mitchel EF, Simonsen L, Griffin MR (1998) Impact of Influenza on Acute Cardiopulmonary Hospitalizations in Pregnant Women. American Journal of Epidemiology 148: 1094 –1102.
2. Blanchard-Rohner G, Siegrist C-A (2011) Vaccination during pregnancy to prevent infections in the neonate? Vaccine 29: 7542–7550. doi:10.1016/j.vaccine.2011.08.013.
3. Skowronska DM, De Serres G (2009) Is routine influenza immunization warranted in early pregnancy? Vaccine 27: 4754–4760. doi:10.1016/j.vaccine.2009.03.079.
4. Harris PW (1998) Influenza Occurring in Pregnant Women. The Journal of the American Medical Association 72: 978 –980. doi:10.1001/jama.1998.016014002.00002.
5. Freeman DW, Barno A (1959) Deaths from Asian Influenza Associated with Pregnancy. Am J Obstet Gynecol 78: 1172–1175.
6. Strategic Advisory Group of Experts on Immunization - report of the extraordinary meeting on the influenza A (H1N1) 2009 pandemic, 7 July 2009 - 2009. Wiley Epidemiol Rec 84: 301–304.
7. Lutren JM, Dolk H, Marnoch GJ (2011) Differences in pandemic influenza vaccination policies for pregnant women in Europe. BMC Public Health 11: 819. doi:10.1186/1471-2458-11-819.
8. Siston AM, Rasmussen SA, Honein MA, Fry AM, Selb K, et al. (2010) Pandemic 2009 influenza A(H1N1) virus illness among pregnant women in the United States. JAMA 303: 1517–1525. doi:10.1001/jama.2010.479.
9. Yates L, Pierce M, Stephens S, Mill AC, Spark P, et al. (2010) Influenza A/ H1N1v in pregnancy: an investigation of the characteristics and management of affected women and the relationship to pregnancy outcomes for mother and infant. Health Technol Assess 14: 109–182 doi:10.3310/hta14340-00.
10. Critical illness due to 2009 A/H1N1 influenza in pregnant and postpartum women: population based cohort study (2010). BMJ 340: c1279.
11. Pierce M, Kurinczuk JJ, Spark P, Broekhuisen P, Knight M, et al. (2011) Perinatal outcomes after maternal 2009/H1N1 infection: national cohort study. BMJ 342: 3321–3324. doi:10.1136/bmj.d3324.
12. Jamieson DJ, Theiler RN, Rasmussen SA (2006) Emerging infections and pregnancy. Emerging Infect Dis 12: 1638–1643.

PLOS ONE | www.plosone.org 9 April 2013 | Volume 8 | Issue 4 | e56700
32. Maternal Immune Response and Neonatal Seroprotection From a Single Dose

31. Zuccotti G PL (2010) TRansplacental antibody transfer following maternal

29. Clark TW, Pareek M, Hoschler K, Dillon H, Nicholson KG, et al. (2009) Trial

28. O’Hagan DT, Rappuoli R, De Gregorio E, Tsai T, Del Giudice G (2011) MF59

27. Stephenson I, Bugarini R, Nicholson KG, Podda A, Wood JM, et al. (2005) Guideline On Dossier Structure And Content For Pandemic Influenza Vaccine

26. Rowe T, Abernathy RA, Hu-Primmer J, Thompson WW, Lu X, et al. (1999) Antibody

25. Guideline On Dossier Structure And Content For Pandemic Influenza Vaccine

20. Følsgaard NV, Chawes BL, Rasmussen MA, Bischoff AL, Carson CG, et al. (2012) Neonatal Cytokine Profile in the Airway Mucosal Lining Fluid Is Skewed by Maternal Atopy. Am J Respir Crit Care Med 185: 275–280. doi:10.1164/rccm.201108-1471OC.

18. Nasidi A, Monath TP, Vandenberg J, Tomori O, Calisher CH, et al. (1993) Cross-Reactivity to Highly Pathogenic Avian Influenza H5N1 Viruses after Vaccination Response in Pregnant Women

17. Schlaudecker EP, McNeal MM, Dodd CN, Ranz JB, Steinhoff MC (2012) Safety of MF59-adjuvanted A/H1N1 influenza vaccine in pregnancy: a comparative cohort study. Am J Obstet Gynecol 207: 177.e1–8. doi:10.1016/j.ajog.2012.07.007.

16. Murray DL, Imagawa DT, Okada DM, St Geme JW Jr (1979) Antibody immunological diagnostic. U.S Dept. Hlth. Ed. Welfare, P.H.S. Atlanta,

15. Hatz C, Von Sonnenburg F, Casula D, Lattanzi M, Leroux-Ronet G (n.d.) Randomized Clinical Trial to Identify the Optimal antigen and MF59 Adjuvant Dose of a Monovalent a/H1N1 Pandemic Influenza Vaccine in Adults and Elderly Subjects. in press.

13. Tamma PD, Ault KA, Del Rio C, Steinhoff MC, Halsey NA, et al. (2009) Safety of maternal influenza vaccination and effect on influenza virus infection in young infants. Arch Pediatr Adolesc Med 165: 104–111. doi:10.1001/archpediatrics.2010.192.

12: 789–796.

10. Impact of prior or concomitant seasonal influenza vaccination on MF59-adjuvanted H1N1v vaccine (Focetria) in adult and elderly subjects. Int J Clin Pract 64: 432–438. doi:11.1111/j.1741-4237.2009.02398.x.

9. Fever vaccination and pregnancy: a four-year prospective study. Trans R Soc Trop Med Hyg 87: 337–339

8. Oster ME, Riehle-Colarusso T, Alverson CJ, Correa A (2011) Associations

7. Stephenson I, Bugarini R, Nicholson KG, Podda A, Wood JM, et al. (2005) Immunogenicity of a Monovalent a/H1N1 Pandemic Influenza Vaccine Among Pregnant Women. Epidemiologic Reviews 28: 47 –53. doi:10.1093/epirev/mx002.

6. Malek A, Sager R, Kuhn P, Nicolaides KH, Schneider H (1996) Evolution of maternofetal transport of immunoglobulins during human pregnancy. Am J Reprod Immunol 36: 245–255.

5. Fox KA, Theiler R (2011) Vaccination in pregnancy. Curr Pharm Biotechnol

4. Heikkinen T, Young J, Van Beek E, Franke H, Verstraeten T, et al. (2012) Safety of MF59-adjuvanted A/H1N1 influenza vaccine in pregnancy: A comparative cohort study. Am J Obstet Gynecol 207: 177.e1–8. doi:10.1016/j.ajog.2012.07.007.

3. Følsgaard NV, Chawes BL, Rasmussen MA, Bischoff AL, Carson CG, et al. (2012) Neonatal Cytokine Profile in the Airway Mucosal Lining Fluid Is Skewed by Maternal Atopy. Am J Respir Crit Care Med 185: 275–280. doi:10.1164/rccm.201108-1471OC.

2. Forcinita in adult and elderly subjects. Int J Clin Pract 64: 432–438. doi:11.1111/j.1741-4237.2009.02398.x.

1. Forcinita in adult and elderly subjects. Int J Clin Pract 64: 432–438. doi:11.1111/j.1741-4237.2009.02398.x.

Vaccination Response in Pregnant Women

Pregnancy modifies the antibody response to trivalent influenza immunization. J Infect Dis 203: 1301–1308. doi:10.1093/infdis/jir026.

Reports of a Controlled Study During an Outbreak of Asian Influenza. Obstet Gynecol 50: 22–29. doi:10.1097/00006282-195001000-00011.

vaccination and effect on influenza virus infection in young infants. Arch Pediatr Adolesc Med 165: 104–111. doi:10.1001/archpediatrics.2010.192.

Safety of MF59-adjuvanted A/H1N1 influenza vaccine during pregnancy and fetal and neonatal outcomes. Am J Public Health 102: e33–40. doi:10.2105/AJPH.2011.300606.

Safety of MF59-adjuvanted A/H1N1 influenza vaccine in pregnancy: a comparative cohort study. Am J Obstet Gynecol 207: 177.e1–8. doi:10.1016/j.ajog.2012.07.007.

Deborah AE, Liu N, Yasseen AS 3rd, Wen SW, et al. (2012) H1N1 influenza vaccine during pregnancy and fetal and neonatal outcomes. Am J Public Health 102: e33–40. doi:10.2105/AJPH.2011.300606.

Safety of MF59-adjuvanted A/H1N1 influenza vaccine in pregnancy: a comparative cohort study. Am J Obstet Gynecol 207: 177.e1–8. doi:10.1016/j.ajog.2012.07.007.

Deborah AE, Liu N, Yasseen AS 3rd, Wen SW, et al. (2012) H1N1 influenza vaccine during pregnancy and fetal and neonatal outcomes. Am J Public Health 102: e33–40. doi:10.2105/AJPH.2011.300606.

Safety of MF59-adjuvanted A/H1N1 influenza vaccine in pregnancy: a comparative cohort study. Am J Obstet Gynecol 207: 177.e1–8. doi:10.1016/j.ajog.2012.07.007.

Deborah AE, Liu N, Yasseen AS 3rd, Wen SW, et al. (2012) H1N1 influenza vaccine during pregnancy and fetal and neonatal outcomes. Am J Public Health 102: e33–40. doi:10.2105/AJPH.2011.300606.

Deborah AE, Liu N, Yasseen AS 3rd, Wen SW, et al. (2012) H1N1 influenza vaccine during pregnancy and fetal and neonatal outcomes. Am J Public Health 102: e33–40. doi:10.2105/AJPH.2011.300606.

Deborah AE, Liu N, Yasseen AS 3rd, Wen SW, et al. (2012) H1N1 influenza vaccine during pregnancy and fetal and neonatal outcomes. Am J Public Health 102: e33–40. doi:10.2105/AJPH.2011.300606.

Deborah AE, Liu N, Yasseen AS 3rd, Wen SW, et al. (2012) H1N1 influenza vaccine during pregnancy and fetal and neonatal outcomes. Am J Public Health 102: e33–40. doi:10.2105/AJPH.2011.300606.

Deborah AE, Liu N, Yasseen AS 3rd, Wen SW, et al. (2012) H1N1 influenza vaccine during pregnancy and fetal and neonatal outcomes. Am J Public Health 102: e33–40. doi:10.2105/AJPH.2011.300606.

Deborah AE, Liu N, Yasseen AS 3rd, Wen SW, et al. (2012) H1N1 influenza vaccine during pregnancy and fetal and neonatal outcomes. Am J Public Health 102: e33–40. doi:10.2105/AJPH.2011.300606.