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Miller, P.D.E., de Silva, T.I. orcid.org/0000-0002-6498-9212, Leonard, H. et al. (6 more authors) (2019) A comparison of viral microneutralization and haemagglutination inhibition assays as measures of seasonal inactivated influenza vaccine immunogenicity in the first year after reduced intensity conditioning, lymphocyte depleted allogeneic haematopoietic stem cell transplant. Vaccine, 37 (3). pp. 452-457. ISSN 0264-410X

https://doi.org/10.1016/j.vaccine.2018.11.061

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A comparison of viral microneutralization and haemagglutination inhibition assays as measures of seasonal inactivated influenza vaccine immunogenicity in the first year after reduced intensity conditioning, lymphocyte depleted allogeneic haematopoietic stem cell transplant.

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Introduction

Respiratory viruses (RV) are identified in approximately 3.5% of haematopoietic stem cell transplant (HSCT) recipients. Influenza A and B viruses account for 18-44% of RV infections [1,2]. Patients transplanted during the influenza season are at highest risk of infection. Progression to pneumonia occurs more frequently in the early post-HSCT period and is associated with a 30-day mortality rate up to 28% [3,4]. Annual administration of a seasonal inactivated influenza vaccine (SIIV) is considered a moderately effective strategy for prevention of infection and influenza-associated hospital admission in the general population[5]; vaccine effectiveness ranging from 19-60% across all age groups has been estimated in the United States over the last decade[6,7]. Vaccine efficacy of 65.4-80% has been reported in HSCT patients, although in small cohorts [8,9], and current guidelines for influenza vaccination of HSCT recipients are largely based on immunogenicity studies.

Historically, the European Medicines Agency (EMA) Committee for Medicinal Products for Human Use (CHMP) immunogenicity criteria for annual SIIV licensing have focussed primarily on rates of seroconversion (defined as a fourfold increase in

antibody (Ab) titre from baseline) and seroprotection (an Ab titre ≥40) detected by serological haemagglutination inhibition (HAI) techniques[10]. Studies in HSCT populations evaluating response to SIIV against CHMP criteria have reported minimal immunogenicity when administered in the first 6 months post-HSCT and impaired response up to at least 12 months post-HSCT[11–13]. So, although HSCT recipients in the early post-HSCT are at high risk of influenza related morbidity and mortality, especially if transplanted during the influenza season, current evidence is insufficient to recommend SIIV administration before at least 4 to 6 months post-HSCT [14,15]. However, in practice a proportion of allogeneic HSCT centres in the United Kingdom (UK) administer the influenza vaccine at earlier time points[16]. Current guidelines do not recommend modification of vaccination schedules according to underlying disease, conditioning intensity, graft manipulation or stem cell source.

A growing body of evidence argues that an HAI Ab titre ≥ 40 may not be seroprotective in population subgroups, and suggests that probability of protection may be better considered along a continuum of titres rather than against this cut-off value[17–19]. In 2016, the CHMP immunogenicity criteria were updated to reflect this, and now a more diverse range of assessment methods of SIIV immune responses including neutralizing Ab titres are recommended [20]. The virus microneutralization (VMN) assay is a highly sensitive and specific method for detecting influenza strain-specific, functional antibodies that inhibit virus entry or block virus replication[21]. The VMN has higher sensitivity than HAI for the detection of low-titre seroconversion particularly to influenza B[22,23] and 2009 pandemic H1N1 virus[24]. Ab titres detectable by VMN assay may confer clinical

protection against influenza virus, although titres have not yet been correlated with clinical efficacy. To our knowledge, the VMN has not previously been used to determine SIIV response in HSCT recipients.

The primary aim of this study was to assess, by HAI and VMN techniques, the immunogenicity of SIIV administered within the first 12 months in a homogenous cohort of reduced intensity conditioning (RIC) peripheral blood stem cell (PBSC) HSCT recipients. The secondary aim was to determine whether in patients vaccinated at less than 3 months, a response is detectable by VMN.

Materials and Methods

Participants

Participants were screened for study eligibility during routine outpatient clinic appointments between October 2015 and February 2016. Eligible participants were aged 16 or over, and recipients of reduced intensity conditioning (RIC) peripheral blood stem cell (PBSC) alloHSCT within 0 and 12 months of transplant. All participants were vaccinated in accordance with standards of care at their transplant centre and were deemed suitable to receive the SIIV by their lead transplant physician. Standard of care at one study centre was to vaccinate from 3 months post-HSCT, while at the other, vaccination was offered at the beginning of the influenza season regardless of time-point post-HSCT. All patients gave written

informed consent. The study was approved by the Health Research Authority National Research Ethics Committee of the UK (Reference 15/YH/0394).

Vaccination and Blood Samples

Patients received in the deltoid muscle, a single injection of a split virion, trivalent 2015-2016 northern hemisphere SIIV (Sanofi-Pasteur, Guildford, UK), containing 15µg haemagglutinin (HA) of each of A/California/7/2009(H1N1)pdm09, A/Switzerland/9715293/2013(H3N2) and B/Phuket/3073/2013. Blood samples were collected at recruitment prior to vaccination, and at approximately four weeks post-vaccination. Serum samples were stored at -20°C until analysis.

Viruses, erythrocytes and cell culture

For VMN assays, live, egg-grown influenza A/California/7/2009(H1N1)pdm09, influenza A/Switzerland/9715293/2013(H3N2) and influenza B/Phuket/3073/2013 (Public Health England, London, UK) were used. HAI assays used the same live, egg-grown influenza A viruses, but ether-treated influenza B virus (Public Health England). A 0.5% solution of turkey erythrocytes in phosphate buffered saline (PBS) (Gibco, Hemel Hempstead, UK) for A(H1N1)pmd09 and B(Phuket), or guinea-pig erythrocytes, for A(H3N2), were used in HAI assays. All VMN assays used Madin-Darby canine kidney (MDCK)(Public Health England, London, UK) cells cultured in Earle's Minimum Essential Medium (MEM) with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and L-Glutamine (Gibco), and supplemented

89 with 0.5mg/ml gentamycin, non-essential amino acids solution (Gibco) and 10% fetal
90 calf serum (FCS) (Gibco).

91

92 *Haemagglutination Inhibition (HAI) and Viral Miconeutralisation (VMN) Assays*

93 All assays were performed in the Public Health England (PHE) respiratory virus
94 reference laboratory. For each of A(H1N1)pdm09, A(H3N2) and B(Phuket), an HAI as
95 previously described [25], and a 3-day VMN assay with modified cytopathic effect
96 (CPE) endpoint, were performed on paired pre- and post-vaccination serum samples.
97 In addition, for A(H1N1)pdm09 a 2-day VMN enzyme-linked immunosorbent (ELISA)
98 assay was performed. In brief, for the CPE-VMN, serum samples heat treated at 56°C
99 for 30 minutes were diluted to 1:10 with PBS followed by serial doubling dilutions
100 across a 96-well u-bottom plate to a dilution of 1:5120. Live egg-grown virus solution
101 (Public Health England, London, UK) standardized to 100x50% tissue culture infective
102 dose/ml (TCID50) was added to each well containing serum, and incubated at 37°C in
103 5% humidified CO_2 atmosphere for 60 minutes. After incubation, confluent MDCK
104 cells in a 96-well culture plate were inoculated with serum-virus mixture, and viral
105 growth medium (VGM) consisting of serum-free, modified Eagle Medium (SF-
106 MEM)(Gibco, Hemel Hempstead, UK), and 1µg/ml Tosyl-phenylalanyl-chloromethyl-
107 ketone(TPCK) treated Trypsin(Sigma, Gillingham, UK) was added. Inoculated cell
108 culture plates were incubated for either 2 hours (influenza A viruses) or 3 hours
109 (influenza B virus) at 37°C in 5% humidified CO_2 atmosphere. Virus-serum inoculum
110 was then aspirated, and each well rinsed twice with 200µl SF-MEM. one-hundred
111 and fifty microlitres VGM (1.0µg/ml for influenza A viruses, 1.5µg/ml for influenza B

virus) was added to wells inoculated with virus, and plates were incubated at 37C°C in 5% humidified CO_2 atmosphere (70 hours for influenza A viruses, 46 hours for influenza B virus). Fifty microlitres of cell supernatant was transferred to corresponding wells of a 96 well v-bottom plate and tested for influenza virus by HA assay. Ab titres were recorded as the reciprocal of the highest dilution at which agglutination was absent. For the A(H1N1)pdm09 ELISA-VMN a virus-serum mixture was prepared as above and added to a 96-well culture plate. After incubation at 37°C in 5% humidified CO_2 atmosphere for 60 minutes, a $5x10^5$ cell/ml MDCK cell suspension was added to each well. Plates were then incubated for 16 hours at 37°C in 5% humidified CO_2 atmosphere. An ELISA was then performed as previously described[26] .

Statistical Analysis

Continuous variables are reported as median values with ranges. Categorical variables are reported as frequencies and percentages. Immunological data is summarised as pre- and post-vaccination geometric mean titres (GMT), and geometric mean ratios (GMRs) of pre- and post-vaccination titres with 95% confidence intervals. The distribution of Ab titre values was not Gaussian, so paired results were compared with the Wilcoxon signed-rank test for non-parametric data. Correlation between GMT and GMRs, and continuous explanatory variables were explored with Spearman's Rank Correlation; for categorical explanatory variables Mann-Whitney test was used. Frequencies of seroconversion and HAI Ab titres ≥ 40 are reported, and the relationship between these outcome measures and categorical

explanatory variables was explored with Pearson's Chi-Square test, or Fisher's exact test; binary logistic regression was used for continuous explanatory variables. The relationship between log10 transformed HAI and VMN titres was explored using a linear regression model. Analysis was performed with IBM SPSS version 24.

Results

Study Population

Twenty-eight alloHSCT recipients with a median age of 57.8 (range 38.0-72.1) years were recruited for the study (Table 1). Participants were vaccinated at a median time-point of 78.5 (range 24-363) days after HSCT, and all 28 gave post-vaccination blood samples at a median of 28 days (range 21-50). All but 1/28 patients provided their sample within the 21 – 28 day window. Donor type was volunteer unrelated donor (VUD) in 71.4% of HSCTs, and sibling in 28.6%. All conditioning regimens included in-vivo lymphocyte depletion with alemtuzumab (89.3%) or antithymocyte globulin (ATG) (10.7%). Graft-versus-host disease (GvHD) was presnt in 28.6% of participants, which was limited to stage 1 acute skin GVHD(17.9%) or mild chronic skin GVHD (10.7%) in all cases. A minority of of participants had been treated with rituximab (10.7%) or intravenous immunoglobulin (7.1%) in the last 12 months.

SIIV Immunogenicity

Geometric mean titres (GMT) and geometric mean ratios (GMR)

GMTs and GMRs of pre- and post-vaccination titres of A(H1N1)pdm09, A(H3N2) and B(Phuket) Ab are shown in Table 2. The GMTs of A(H1N1)pdm09 and A(H3N2) Ab were higher by CPE-VMN than HAI, and A(H1N1) GMTs were higher by ELISA-VMN than CPE-VMN, at both pre- and post-vaccination timepoints. However, none of the three assays detected a significant change in Ab titre following vaccination, as reflected in GMRs of pre- and post-vaccination Ab titres close to 1. In contrast, B(Phuket) GMTs were higher by HAI than CPE-VMN at both pre- and post-vaccination timepoints. Despite vaccination, there was a statistically significant decline in B(Phuket)-specific Ab titres from pre- to post- vaccination by both HAI (15.17 v 11.89, p=0.017) and CPE-VMN (6.98 v 6.25, p=0.018) with similar GMRs of 0.78 (95% CI 0.62-0.94) and 0.89 (0.82-0.96) by both assays.

Frequency of detectable Ab titres and frequency of seroconversions

Considering both pre- and post-vaccination values, 25/56 (44.6%) serum samples had detectable Ab against H1N1(pdm09) by HAI compared with 31 (55.4%) by CPE-VMN and 38 (67.9%) by ELISA-VMN. For H3N2, 32 (57.1%) serum samples had detectable Ab by HAI versus 54 (96.4%) by VMN, while for B(Phuket) equivalent values are 27 (48.2%) by HAI versus 12 (21.4%) by VMN. No seroconversions to any vaccine component were detected by HAI assay or CPE-VMN. A single seroconversion was detected by ELISA-VMN in a patient vaccinated at 9 weeks post-HSCT.

HAI titres ≥ 40

The frequency of patients with HAI Ab titres ≥ 40 are displayed by vaccination time-point in Table 3. Pre-vaccination, 50% of participants had HAI Ab titres ≥ 40 against any single vaccine component. Frequency of HAI Ab titre ≥ 40 was highest in those vaccinated at 0-3 months (60.0%) and lowest at 6-12 months (28.6%) although this trend was not statistically significant (p=0.39). As seroprotective titres for CPE and ELISA-VMN have not been defined, equivalent data are not presented for these assays.

Relationship between HAI and VMN titres

Statistically significant correlation was observed between HAI and VMN titres for all 3 strains (p<0.001). From the linear regression equation, CPE-VMN titres equivalent to an HAI titre of 40 were estimated as 65.18 (95%CI 42.33-100.36) for A(H1N1)pdm09, 366.77 (95% CI 105.41-1276.12) for A(H3N2), and 10.17 (95% CI 7.74-13.36) for B(Phuket). For A(H1N1)pdm09, the ELISA-VMN titre equivalent to an HAI titre of 40 was 164.10 (95% CI 86.37-311.78).

Discussion

In this study, the immunogenicity of the 2015-2016 SIIV was evaluated in HSCT recipients using the HAI and VMN assays. This is the first study to report VMN data

in this patient group. A limitation of this study is that it did not include a comparator arm of immunocompetent participants.

GMTs for A(H1N1)pdm09 and A(H3N2) determined by VMN were statistically significantly higher than by HAI, suggesting VMN may provide a more sensitive assay to detect influenza-specific antibody titres in this population. The estimated VMN equivalent of an HAI titre of 40 was 65.18 (95% CI 42.33-100.36) for CPE endpoint and 164.10 for ELISA endpoint . Previous studies of H1N1(A/Brisbane/59/2007) in a paediatric population, and A(H1N1)pdm09 in a healthy adult population using an ELISA-based VMN, estimated that titres of 200 and 211 respectively were equivalent to an HAI titre of 40[27,28]. The same paediatric study estimated that VMN titre of 140 was equivalent to HAI titre of 40 for H3N2(A/Brisbane/10/2007). In a small study of patients infected by H3N2(A/SouthAfrica/114/95/7), GMTs by HAI and ELISA-based VMN were 29.19 and 362.98 respectively[29]. The comparative A(H3N2) HAI and VMN titres in this present study are similar to these previous findings, while for A(H1N1)pdm09 our estimate is lower. For A(H1N1)pdm09, an ELISA-based VMN appears to offer greater sensitivity than a CPE-VMN for detection of strain-specific Ab. A(H3N2) viruses have been the dominant circulating strains and a component of the SIIV since at least 1998[30], while the A(H1N1)pdm09 virus is by definition antigenically dissimilar to H1N1 strains preceding 2009. The presence of cross-reacting neutralizing Ab to A(H3N2) from previous exposure may explain why the titres by VMN were markedly higher than for the more recent A(H1N1)pdm09 virus.

For B(Phuket) the VMN assay GMT was statistically significantly lower than the HAI titre. The estimated equivalent VMN titre of HAI 40 was 10.17 (95% CI 7.74-13.36). A previous study comparing HAI and VMN reported increased rates of seroconversion by VMN compared with HAI but equivalent GMTs were not reported[23]. Several previous studies have documented low sensitivity of the HAI assay when using influenza B virus, which can partially be overcome by ether-treatment of the antigen[31–33]. Ether treatment cleaves the virion and increases Ab binding sites[32,34] however the virion is rendered unable to replicate and therefore unsuitable for use in VMN assay. We used the same egg-grown B(Phuket) virus batch in both HAI and VMN assays to improve comparability of the data, and applied antigen modification with ether treatment to the part of the virus batch to be used in the HAI to optimise assay sensitivity. This may account for the relative insensitivity of VMN compared with the HAI in our study.

In this study population, seroconversion by HAI was completely absent for all 3 vaccine components, while the more sensitive ELISA-VMN detected a single seroconversion in a patient vaccinated within the first 3 months of HSCT. Pauksen et al. observed seroconversion rates by HAI to SIIV administered within 12 months post-HSCT of 31% for A(H1N1), 9% for A(H3N2), and 20% for influenza B. Conditioning intensity, HSC source and use of lymphocyte depletion were not reported[12]. Karras et al reported low seroconversion rates of 0% to A(H3N2), 6% for A(H1N1) and 8% for Influenza B[13]. In this study 44.6% of patients received RIC and the remainder myeloablative conditioning (MAC) and none received lymphocyte deplete grafts. In bone marrow alloHSCT recipients who universally received

lymphocyte deplete grafts, no seroconversions were reported in the first 6 month post-HSCT[11]. In our present study, universal in-vivo lymphocyte depletion may have impacted on vaccine immunogenicity. Both alemtuzumab and ATG are broadly immunosuppressive with activity beyond the target T-cell compartment[35], and In vivo lymphocyte depletion with these agents may contribute to delayed immune reconstitution and an increased risk of viral infection[36,37]. In the solid organ transplant setting, a trend towards poorer response to SIIV in patients vaccinated within a year of receiving ATG has been reported[38]. The median age in our study was 57.8, compared with 21-40.8 [11,13] in the studies above. Older age is associated with poorer influenza vaccine immunogenicity in the general population and this may have been a contributing factor to the poor response in this study population[39].

Despite vaccination, rates of titre ≥ 40 by HAI were stable from pre- to post-vaccination for A(H3N2) and fell for A(H1N1) and B(Phuket). Baseline seroprotection rates were 28.6% for A(H1N1)pdm09, 14.3% for A(H3N2), 32.1% for B(Phuket) and 50% to any 1 or more strain. In an immunogenicity study of the monovalent A(H1N1)pdm09 vaccine, Issa and colleagues reported seroresponse rates to the study vaccine, but also HAI titres ≥ 40 to the seasonal influenza strains. These ranged from 20.7% for Influenza B to 57.4% for A(H3N2). However, these patients were evaluated at 2.5 to 92.7 months post-HSCT, and some had received the seasonal IIV in previous post-HSCT influenza seasons. In contrast, patients in this current study were all seasonal IIV naïve following HSCT. Other studies have reported baseline seroprotection rates to Influenza A and B of 12-16%[12] and 0-

29%[40]. Pre-vaccination rates of HAI titre ≥ 40 fell with time from HSCT (60% at 0-3 months, 50% at 3-6 months, 28.6% at 6-12 months) and this is consistent with previous studies that have reported a waning of disease specific Ab within the first-year post-HSCT. Although we did not compare pre- with post-HSCT titres, our findings suggest that pre-HSCT vaccination may be an approach to protecting recipients during the first few months post-HSCT when they are most vulnerable. A study investigating this approach has shown seroresponse rates of 22.9% (H1N1) and 25% (H3 and B Ag) when recipients were vaccinatd 10 days pre-HSCT[41].

None of the evaluated patient characteristics correlated with seroresponse measures or with GMT or GMRs (data not shown). Neither active GvHD nor concomitant IST correlated with post-vaccination HAI titre ≥ 40. An association between IST, GvHD and response to influenza vaccination has not been identified consistently. Our findings are in agreement with previous studies reporting low response by HAI in the first 12 months. While Karras and colleagues suggest that equivalent seroconversion rates to 1 or more strains at 2-6 and 6-12 months (12 % v 30% p=0.43)[13] may justify early vaccination, our findings of almost entirely absent humoral response throughout the first year would argue against this strategy in RIC PBSC lymphocyte deplete alloHSCT recipients.

Conlusions

In conclusion, this is the first study to use the VMN assay to assess the immunogenicity of seasonal IIV in HSCT recipients. The CPE and ELISA VMN detected

289 Ab in more serum samples than HAI, and GMTs were statistically significantly higher
290 by VMN than HAI for A(H1N1)pdm09 and A(H3N2). However, for influenza B, GMTs
291 were lower by VMN than an ether-modified HAI. The ELISA-VMN detected a single
292 seroconversion to A(H1N1)pdm09. This limited seroresponse to trivalent SIIV
293 administered in the first-year post-HSCT in a cohort of RIC PBSC alloHSCT recipients
294 who underwent in-vivo lymphocyte depletion suggests that a more tailored
295 approach to vaccination may be desirable, although future studies to define clinical
296 and immunological predictors of response to vaccine are required. Furthermore,
297 there is a clear need for novel immunogenic vaccination schedules and vaccine
298 formulations in this patient group. Early phase studies of high-dose seasonal
299 influenza vaccines have shown promising results[42]. In line with CHMP
300 recommendations, consideration should be given to using the VMN assay to assess
301 immunological response to SIIV in such future studies, and combining this with
302 clinical efficacy data may define seroprotective VMN titres. The VMN assay may
303 provide useful data in other immunocompromised patient groups such as recipients
304 of chemo- or immunotherapies and future studies are warranted.

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Table 1. Characteristics of n=28 Study Participants

Characteristic (n=28)	Value
Age at HSCT, median (range), (IQR)	57.8 (38.0-72.1), (12.7)
Gender male, n(%)	15 (53.6)
Diagnosis, n (%)	
Acute lymphoblastic leukaemia (ALL)	3 (10.7)
Acute myeloid leukaemia (AML)	14 (50.0)
Chronic lymphocytic leukaemia (CLL)	1 (3.6)
Chronic myelomonocytic leukaemia (CMML)	1 (3.6)
Myelodysplastic syndrome (MDS)	4 (14.3)
Myelofibrosis (MF)	2(7.1)
Multiple myeloma (MM)	1 (3.6)
Non-Hodgkin Lymphoma (NHL)	2 (7.1)
Donor type, n (%)	
Sibling donor	8 (28.6)
Volunteer unrelated donor (VUD)	20 (71.4)
Stem cell source, n (%)	
Peripheral blood stem cell (PBSC)	28 (100)
Conditioning Intensity, n (%)	

Reduced intensity	28 (100)
Lymphocyte depletion, n (%)	
Alemtuzumab	25 (89.3)
Antithymocyte globulin (ATG)	3 (10.7)
Days from HSCT to vaccination, median (range), (IQR)	78.5 (24-363), (136)
Months from HSCT to vaccination, n (%)	
0-3	15(53.6)
>3-6	6 (21.4)
>6-12	7 (25)
Lymphocyte count (x10^9) at vaccination, median (range), (IQR)	0.57 (0.02-2.98),(0.63)
Graft versus host disease at vaccination, n(%)	8 (28.6)
Acute (stage 1, skin)	5 (17.9)
Chronic (mild, skin)	3 (10.7)
Immunosuppressive therapy (IST) at vaccination, n(%)	
Any IST	18 (64.3)
Single agent	13 (46.4)
Dual agent	4 (14.3)
Triple agent	1 (3.6)
Intravenous Immunoglobulin (IVIg) in last 12 months, n(%)	2 (7.1)
Rituximab in last 12 months, n(%)	3 (10.7)

Table 2. Geometric Mean Titres (GMT), Geometric Mean Ratios (GMR), percentage of seroconversions and percentage of titres ≥40 of A(H1N1)pdm09, A(H3N2) and B(Phuket) antibodies by Haemagglutination (HAI) and Virus Microneutralisation (VMN) assays. Value (95% Confidence interval).

	Vaccine Component		
	A(H1N1)pdm09	**A(H3N2)**	**B(Phuket)**
HAI			
GMT pre-vaccination	12.65 (7.94-21.67)	11.46 (7.76-18.05)	15.17 (9.31-35.41)
GMT post-vaccination	11.45 (7.44-19.06)	11.60 (8.07-17.89)	11.89 (7.54-20.50)
GMR	0.91 (0.78-1.03)	1.01 (0.93-1.11)	0.78(0.62-0.94)
% seroconversion	0	0	0
% Pre-vaccination HAI ≥40	28.6 (8)	14.3 (4)	32.1 (9)
% Post Vacc HAI ≥40	25.0 (7)	14.3 (4)	25.0 (7)
CPE-VMN			
GMT pre-vaccination	16.82 (9.26-33.22)	129.64(70.62-241.87)	6.98 (5.54-9.14)
GMT post-vaccination	16.41 (9.76-30.55)	118.88 (67.68-212.68)	6.25 (5.20-7.69)
GMR	0.98 (0.82-1.17)	0.92 (0.74-1.11)	0.89 (0.82-0.96)
% seroconversion	0	0	0
ELISA-VMN			
GMT pre-vaccination	34.43 (16-85-75.68)		
GMT post-vaccination	32.87 (16.80-68.49)		
GMR	0.95 (0.74-1.11)		
% seroconversion	3.6 (1)		

Table 3. Proportion of patients with pre and post-vaccination haemagglutination inhibition (HAI) antibody (Ab) titres ≥ 40

Vaccine Component	Vaccination timepoint, months	Pre-Vaccination titres ≥40,n (%)	Post-Vaccination, ≥40,n (%)
A(H1N1)pdm09	<3 (n=15)	5 (33.3)	4 (26.7)
	3-6 (n=6)	2 (33.3)	2 (33.3)
	6-12 (n=7)	1 (14.3)	1 (14.3)
	Total	8 (28.6)	7 (25.0)
A(H3N2)	<3 (n=15)	2 (13.3)	2 (13.3)
	3-6 (n=6)	1 (16.7)	1 (16.7)
	6-12 (n=7)	1 (14.3)	1 (14.3)
	Total	4 (14.3)	4 (14.3)
B(Phuket)	<3 (n=15)	6 (40.0)	4 (26.7)
	3-6 (n=6)	1 (16.7)	1 (16.7)
	6-12 (n=7)	2 (28.6)	2 (28.6)
	Total	9 (32.1)	7(25.0)

≥ any one vaccine component	<3 (n=15)	9 (60.0)	9 (60.0)
	3-6 (n=6)	3 (50.0)	3 (50.0)
	6-12 (n=7)	2 (28.6)	2 (28.6)
	Total	14 (50)	14 (50.0)

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