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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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ABSTRACT

Traditionally, immune response to influenza vaccines has been measured using the haemagglutination inhibition (HAI) assay. A broader repertoire of techniques including the sensitive viral microneutralization (VMN) assay is now recommended by the European Medicines Agency (EMA). Comparing HAI and VMN, we determined immune response to a trivalent 2015–2016 seasonal inactivated influenza vaccine (SIIV) administered to 28 recipients of allogeneic haematopoietic stem cell transplant (HSCT). Vaccination was within the first-year post-transplant at a median of 78.5 (24–363) days. The proportion of patients with baseline and post-vaccination HAI titres $\geq$ 1:40 were 28.6% and 25% for A(H1N1)pdm09, 14.3% at both timepoints for A(H3N2), and 32.1% and 25% for B(Phuket). Pre and Post-vaccination geometric mean titres(GMT) were higher by VMN than HAI for A(H1N1)pdm09 and A(H3N2), but lower for B(Phuket) ($p=<0.05$). Geometric mean ratios(GMR) of baseline and post-vaccination titres were similar by HAI and VMN($p > 0.05$) for all components. A single seroconversion to A(H1N1) was detected by ELISA-VMN. None of patient age, lymphocyte count, days from transplant to vaccination, donor type, or graft-versus-host disease (GVHD) or immunosuppressive therapy (IST) at vaccination correlated with baseline or post-vaccination titres by either assay. This absence of seroresponse to SIIV in the first-year post HSCT highlights the need for novel immunogenic vaccination formulations and schedules in this high-risk population.

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1. 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 to 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

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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 $\geq$ 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 period 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 $\geq$ 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. 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.

2. Materials and methods

2.1. 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 RIC PBSC allogeniec HSCT (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).

2.2. 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 15 μg 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.

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

2.4. Haemagglutination inhibition (HAI) and viral miconeutralisation (VMN) assays

All assays were performed in the Public Health England (PHE) respiratory virus reference laboratory. For each of A(H1N1) pdm09, A(H3N2) and B(Phuket), an HAI as previously described [25], and a 3-day VMN assay with modified cytopathic effect (CPE) endpoint, were performed on paired pre- and post-vaccination serum samples. In addition, for A(H1N1)pdm09 a 2-day VMN enzyme-linked immunosorbent (ELISA) assay was performed. In brief, for the CPE-VMN, serum samples heat treated at 56 °C for 30 min were diluted to 1:10 with PBS followed by serial doubling dilutions across a 96-well u-bottom plate to a dilution of 1:5120. Live egg-grown virus solution (Public Health England, London, UK) standardized to 100x50% tissue culture infective dose/ml (TCID50) was added to each well containing serum, and incubated at 37 °C in 5% humidified CO_2 atmosphere for 60 min. After incubation, confluent MDCK cells in a 96-well culture plate were inoculated with serum-virus mixture, and viral growth medium (VGM) consisting of serum-free, modified Eagle Medium (SF-MEM) (Gibco, Hemel Hempstead, UK), and 1 μg/ml Tosyl-phenylalanyl-chloromethyl-ketone (TPCK) treated Trypsin (Sigma, Gillingham, UK) was added. Inoculated cell culture plates were incubated for either 2 h (influenza A viruses) or 3 h (influenza B virus) at 37 °C in 5% humidified CO_2 atmosphere. Virus-serum inoculum was then aspirated, and each well rinsed twice with 200 μl SF-MEM. one-hundred and fifty microlitres VGM (1.0 μg/ml for influenza A viruses, 1.5 μg/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 h for influenza A viruses, 46 h 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 haemagglutination 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 min, a 5×10^5 cell/ml MDCK cell suspension was added to each well. Plates were then incubated for 16 h at 37 °C in 5% humidified CO_2 atmosphere. An ELISA was then performed as previously described [26].

2.5. 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 (GMR) 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.

3. Results

3.1. 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 present 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.

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)

3.2. SIIV immunogenicity

3.2.1. 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.

3.2.2. 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.

3.2.3. 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.

3.3. 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).

Table 2
Geometric Mean Titres (GMT) and Geometric Mean Ratios (GMR) with 95% confidence intervals, 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.

	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)
% (n) seroconversion	0 (0)	0 (0)	0 (0)
% (n) pre-vaccination HAI ≥ 40	28.6 (8)	14.3 (4)	32.1 (9)
% (n) post-vacc HAI ≥ 40 (n)	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)
% (n) seroconversion	0 (0)	0 (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)		
% (n) 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 HAI ≥ 40,n (%)	Post-vaccination, HAI ≥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 (50.0)	14 (50.0)

4. 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 (95% CI 86.37–311.78) 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 a 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 cleaved 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, haematopoietic stem cell source and use of lymphocyte depletion were not reported [12]. Karras et al reported low seroconversion rates of 0%, 6% and 8% for influenza A(H3N2), A(H1N1) and influenza B respectively [13]. In this study 44.6% of patients received RIC and the remainder myeloablative conditioning (MAC) and none received lymphocyte

depleted grafts. In bone marrow alloHSCT recipients who universally received lymphocyte depleted 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 our study population [39].

Despite vaccination, rates of titre $\geq$ 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 $\geq$ 40 to A(H3N2) and influenza B seasonal 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 [43]. 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 $\geq$ 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 optimising Ab titres with 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 $\geq$ 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 depleted alloHSCT recipients.

5. Conclusions

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

Conflicts of interest

No conflicts of interest declared.

References

[1] Ljungman P, Ward KN, Crooks BNa, Parker A, Martino R, Shaw PJ, et al. Respiratory virus infections after stem cell transplantation: a prospective study from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation Summary. Bone Marrow Transplant 2001;28:479–84. https://doi.org/10.1038/sj.bmt.1703139.
[2] Hassan Ia, Chopra R, Swindell R, Mutton KJ. Respiratory viral infections after bone marrow/peripheral stem-cell transplantation: the Christie hospital experience. Bone Marrow Transplant 2003;32:73–7. https://doi.org/10.1038/sj.bmt.1704048.
[3] Nichols WG, Guthrie Ka, Corey L, Boeckh M. Influenza infections after hematopoietic stem cell transplantation: risk factors, mortality, and the effect of antiviral therapy. Clin Infect Dis 2004;39:1300–6. https://doi.org/10.1086/425004.
[4] Whimbey E, Elting LS, Couch RB, Lo W, Williams L, Champlin RE, et al. Influenza A virus infections among hospitalized adult bone marrow transplant recipients. Bone Marrow Transplant 1994;13:437–40.
[5] Osterholm MT, Kelley NS, Sommer A, Belongia Ea. Efficacy and effectiveness of influenza vaccines: A systematic review and meta-analysis. Lancet Infect Dis 2012;12:36–44. https://doi.org/10.1016/S1473-3099(11)70295-X.
[6] Zimmerman RK, Nowalk MP, Chung J, Jackson ML, Jackson LA, Petrie JG, et al. 2014–2015 Influenza vaccine effectiveness in the United States by vaccine type. Clin Infect Dis 2016;63:1564–73. https://doi.org/10.1093/cid/ciw635.
[7] Treanor JJ, Talbot HK, Ohmit SE, Coleman LA, Thompson MG, Cheng P-Y, et al. Effectiveness of seasonal influenza vaccines in the united states during a season with circulation of all three vaccine strains. Clin Infect Dis 2012;55:951–9. https://doi.org/10.1093/cid/cis574.
[8] Ambati A, Boas LSV, Ljungman P, Testa L, De Oliveira JF, Aoun M, et al. Evaluation of pretransplant influenza vaccination in hematopoietic SCT: A randomized prospective study. Bone Marrow Transplant 2015;50:858–64. https://doi.org/10.1038/bmt.2015.47.
[9] Machado CM, Cardoso MRa, da Rocha IF, Boas LSV, Dulley FL, Pannuti CS. The benefit of influenza vaccination after bone marrow transplantation. Bone Marrow Transplant 2005;36:897–900. https://doi.org/10.1038/sj.bmt.1705159.
[10] European Medicines Agency. Note for guidance on harmonisation of requirements for influenza vaccines. 1997.
[11] Engelhard D, Nagler A, Hardan I, Morag A, Aker M, Baciu H, et al. Antibody response to a two-dose regimen of influenza vaccine in allogeneic T cell-depleted and autologous BMT recipients. Bone Marrow Transplant 1993;11:1–5.
[12] Pauksen K, Linde a, Hammarström V, Sjölin J, Carneskog J, Jonsson G, et al. Granulocyte-macrophage colony-stimulating factor as immunomodulating factor together with influenza vaccination in stem cell transplant patients. Clin Infect Dis 2000;30:342–8. https://doi.org/10.1086/313663.
[13] Karras Na, Weeres M, Sessions W, Xu X, DeFor TE, Young J-AAH, et al. A randomized trial of one versus two doses of influenza vaccine after allogeneic transplantation. Biol Blood Marrow Transplant 2013;19:109–16. https://doi.org/10.1016/j.bbmt.2012.08.015.
[14] Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, Storek J, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. Preface. Bone Marrow Transplant 2009;15:1143–238. https://doi.org/10.1038/bmt.2009.254.
[15] Rubin LG, Levin MJ, Ljungman P, Davies EG, Avery R, Tomblyn M, et al. 2013 IDSA clinical practice guideline for vaccination of the immunocompromised host. Clin Infect Dis 2014;58:e44–e100. https://doi.org/10.1093/cid/cit684.
[16] Miller PDE, de Silva TI, Skinner R, Gilleece MH, Peniket A, Hamblin A, et al. Routine vaccination practice after adult and paediatric allogeneic

haematopoietic stem cell transplant: a survey of UK NHS programmes. Bone Marrow Transplant 2017;52(5):775–7. https://doi.org/10.1038/bmt.2016.362.
[17] Coudeville L, Bailleux F, Riche B, Megas F, Andre P, Ecochard R. Relationship between haemagglutination-inhibiting antibody titres and clinical protection against influenza: development and application of a bayesian random-effects model. BMC Med Res Methodol 2010;10:18. https://doi.org/10.1186/1471-2288-10-18.
[18] Barrett PN, Berezuk G, Fritsch S, Aichinger G, Hart MK, El-Amin W, et al. Efficacy, safety, and immunogenicity of a Vero-cell-culture-derived trivalent influenza vaccine: a multicentre, double-blind, randomised, placebo-controlled trial. Lancet (London, England) 2011;377:751–9. https://doi.org/10.1016/S0140-6736(10)62228-3.
[19] Black S, Nicolay U, Vesikari T, Knuf M, Del Giudice G, Della Cioppa G, et al. Hemagglutination inhibition antibody titers as a correlate of protection for inactivated influenza vaccines in children. Pediatr Infect Dis J 2011;30:1081–5. https://doi.org/10.1097/INF.0b013e3182531f47.
[20] European Medicines Agency. Guideline on Influenza Vaccines 2014;44.
[21] Katz JM, Hancock K, Xu X. Serologic assays for influenza surveillance, diagnosis and vaccine evaluation. Expert Rev Anti Infect Ther 2011;9:669–83. https://doi.org/10.1586/eri.11.51.
[22] Kendal aP, Cate TR. Increased sensitivity and reduced specificity of hemagglutination inhibition tests with ether-treated influenza B/Singapore/222/79. J Clin Microbiol 1983;18:930–4.
[23] Frank aL, Puck J, Hughes BJ, Cate TR. Microneutralization test for influenza A and B and parainfluenza 1 and 2 viruses that uses continuous cell lines and fresh serum enhancement. J Clin Microbiol 1980;12:426–32.
[24] Veguilla V, Hancock K, Schiffer J, Gargiullo P, Lu X, Aranio D, et al. Sensitivity and specificity of serologic assays for detection of human infection with 2009 pandemic H1N1 virus in U.S. populations. J Clin Microbiol 2011;49:2210–5. https://doi.org/10.1128/JCM.00229-11.
[25] World Health Organization. Manual for the laboratory diagnosis and virological surveillance of influenza. World Heal Organ 2011;2011:153.
[26] Who. Manual for the laboratory diagnosis and virological surveillance of influenza.. World Heal Organ 2011 2011:153.
[27] Verschoor CP, Singh P, Russell ML, Bowdish DME, Brewer A, Cyr L, et al. Microneutralization assay titres correlate with protection against seasonal influenza H1N1 and H3N2 in children. PLoS One 2015;10:e0131531. https://doi.org/10.1371/journal.pone.0131531.
[28] Babor F, Grund S, Siepermann M, Oommen PT, Kuhlen M, Schuster FR, et al. Epidemiology and clinical characteristics of pandemic (H1N1) 2009 influenza infection in pediatric hemato-oncology and hematopoietic stem cell transplantation patients. Transpl Infect Dis 2012;14:589–94. https://doi.org/10.1111/tid.12013.
[29] Rowe T, Abernathy Ra, Hu-Primmer J, Thompson WW, Lu X, Lim W, et al. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. J Clin Microbiol 1999;37:937–43.
[30] World Health Organization. Recommendations for Influenza Vaccine. Composition 1998.
[31] Mancini G, Donatelli I, Arangio-Ruiz G, Rozera C, Macchia T. Comparison of haemagglutination-inhibition and single radial haemolysis techniques for detecting antibodies to influenza A and B viruses. J Hyg (Lond) 1983;91:157–62.
[32] Pyhala R, Kleemola M, Viakorpi R. The HI test modified by ether treatment in the sero-epidemiological surveillance of influenza B. J Hyg (Lond) 1985;94:341–8.
[33] Monto AS, Maassab HF. Ether treatment of type B influenza virus antigen for the hemagglutination inhibition test. J Clin Microbiol 1981;13:54–7.
[34] Monto AS, Kendal AP. Effect of neuraminidase antibody on Hong Kong influenza. Lancet (London, England) 1973;1:623–5.
[35] Mohty M. Mechanisms of action of antithymocyte globulin: T-cell depletion and beyond. Leukemia 2007;21:1387–94. https://doi.org/10.1038/sj.leu.2404683.
[36] Chakrabarti S, Mackinnon S, Chopra R, Kottaridis PD, Peggs K, O'Gorman P, et al. High incidence of cytomegalovirus infection after nonmyeloablative stem cell transplantation: potential role of Campath-1H in delaying immune reconstitution. Blood 2002;99.
[37] Barge RMY, Starrenburg CWJ, Falkenburg JHF, Fibbe WE, Marijt EW, Willemze R. Long-term follow-up of myeloablative allogeneic stem cell transplantation using Campath ?in the bag? as T-cell depletion: the Leiden experience. Bone Marrow Transplant 2006;37:1129–34. https://doi.org/10.1038/sj.bmt.1705385.
[38] Orcurto A, Pascual M, Hoschler K, Aubert V, Meylan P, Manuel O. Impact of anti–T-cell therapy in the immunogenicity of seasonal influenza vaccine in kidney transplant recipients. Transplant J 2012;94:630–6. https://doi.org/10.1097/TP.0b013e31825f7f82.
[39] Goodwin K, Viboud C, Simonsen L. Antibody response to influenza vaccination in the elderly: A quantitative review. Vaccine 2006;24:1159–69. https://doi.org/10.1016/j.vaccine.2005.08.105.
[40] Avetisyan G, Aschan J, Hassan M, Ljungman P. Evaluation of immune responses to seasonal influenza vaccination in healthy volunteers and in patients after stem cell transplantation. Transplantation 2008;86:257–63. https://doi.org/10.1097/TP.0b013e3181772a75.
[41] Harris AE, Styczynski J, Bodge M, Mohty M, Savani BN, Ljungman P. Pretransplant vaccinations in allogeneic stem cell transplantation donors and recipients: an often-missed opportunity for immunoprotection? Bone Marrow Transplant 2015;50:899–903. https://doi.org/10.1038/bmt.2015.49.
[42] Halasa NB, Savani BN, Asokan I, Kassim A, Simons R, Summers C, et al. Randomized double-blind study of the safety and immunogenicity of standard-dose trivalent inactivated influenza vaccine versus high-dose trivalent inactivated influenza vaccine in adult hematopoietic stem cell transplantation patients. Biol Blood Marrow Transplant 2016;22:528–35. https://doi.org/10.1016/j.bbmt.2015.12.003.
[43] Issa NC, Marty FM, Gagne LS, Koo S, Verrill KA, Alyea EP, et al. Seroprotective Titers against, H_1N_1 Influenza A Virus after Vaccination in Allogeneic Hematopoietic Stem Cell Transplantation Recipients. Biol Blood Marrow Transplant 2011;17:434–8. https://doi.org/10.1016/j.bbmt.2010.10.002.