

Enhanced Immune Response after a Second Dose of an AS03-Adjuvanted H1N1 Influenza A Vaccine in Patients after Hematopoietic Stem Cell Transplantation

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Seroconversion rates following influenza vaccination in patients with hematologic malignancies after hematopoietic stem cell transplantation (HSCT) are known to be lower compared to healthy adults. The aim of our diagnostic study was to determine the rate of seroconversion after 1 or 2 doses of a novel split virion, inactivated, AS03-adjuvanted pandemic H1N1 influenza vaccine (A/California/7/2009) in HSCT recipients (ClinicalTrials.gov Identifier: NCT01017172). Blood samples were taken before and 21 days after a first dose and 21 days after a second dose of the vaccine. Antibody (AB) titers were determined by hemagglutination inhibition assay. Seroconversion was defined by either an AB titer of $\leq 1:10$ before and $\geq 1:40$ after or $\geq 1:10$ before and ≥ 4 -fold increase in AB titer 21 days after vaccination. Seventeen patients (14 allogeneic, 3 autologous HSCT) received 1 dose and 11 of these patients 2 doses of the vaccine. The rate of seroconversion was 41.2% (95% confidence interval [CI] 18.4-67.1) after the first and 81.8% (95% CI 48.2-97.7) after the second dose. Patients who failed to seroconvert after 1 dose of the vaccine were more likely to receive any immunosuppressive agent ($P = .003$), but time elapsed after or type of HSCT, age, sex, or chronic graft-versus-host disease was not different when compared to patients with seroconversion. In patients with hematologic malignancies after HSCT the rate of seroconversion after a first dose of an adjuvanted H1N1 influenza A vaccine was poor, but increased after a second dose.

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INTRODUCTION

A novel split virion, inactivated, adjuvanted pandemic H1N1 influenza A vaccine (A/California/7/2009 NYMC X-179A; Pandemrix) was licensed in most European countries in 2009. The Centers for Disease Control (CDC) [1] recommended H1N1 in-

fluenza A vaccination for adult individuals, especially for immunocompromised or patients with chronic medical conditions. Patients with hematologic malignancies after hematopoietic stem cell transplantation (HSCT) seem to be at a higher risk of seasonal and H1N1 influenza A-related complications with a reported case fatality of up to 25% for seasonal [2-7] and up to 33% for H1N1 influenza A [8-11]. Protection against influenza is primarily mediated by virus-specific antibodies (AB) and depends on the humoral immune response [12,13], which is impaired in these individuals. Based on data from seasonal influenza, it is assumed that a H1N1 virus-specific antibody titer of $\geq 1:40$ is associated with an approximately 50% lower risk of developing H1N1 influenza A and is therefore referred to as a seroprotective titer [14]. The immunogenicity of seasonal influenza vaccines in patients after HSCT seems lower compared to the general population, especially when vaccinated < 6 months after HSCT (reviewed in [7] and [15]). First reports are also indicating weak response rates to vaccination against the 2009 H1N1 influenza A [16].

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Whether a second dose of an influenza vaccine results in a higher rate of seroconversion is still under debate. Thus, the aim of the present study was to investigate the rate of seroconversion after 1 and 2 doses of a novel H1N1 influenza A vaccine (ClinicalTrials.gov Identifier: NCT01017172).

METHODS

Patients

Adult patients with hematologic malignancies after HSCT from the Medical Department of the Johann Wolfgang Goethe University, who were routinely scheduled to receive a H1N1 influenza A vaccine, were asked to participate in this diagnostic study. The study protocol was approved by the local ethics committee and complied with the ICH-GCP guideline on the conduct of clinical trials and the Declaration of Helsinki. All patients gave written informed consent.

A 10-mL blood sample was taken immediately before (day 0) and 3 weeks after the first dose of the vaccine (day 21). On day 21, a second dose of the vaccine was given, and subsequently, a third blood sample was taken 21 days after the second vaccination on day 42. All blood samples were centrifuged (10 minutes at 1500 r.p.m.), and the sera were frozen (-20°C) for further analysis. Clinical data were retrieved from the medical records.

Vaccine

Split influenza virus, inactivated, containing 3.75- μg antigen equivalent to A/California/7/2009 (H1N1) v-like strain (X-179A) hemagglutinin with AS03 adjuvant composed of squalene (10.69 mg), DL- α -tocopherol (11.86 mg), and polysorbate 80 (4.86 mg) (Pandemrix, GlaxoSmithKline Biologicals, Dresden, Germany) was used for an intramuscular vaccination into the deltoid muscle of the nondominant arm. The second dose was given into the opposite arm.

Hemagglutination Inhibition Test

Hemagglutination inhibition assay (HAI) was done after removing naturally occurring, nonspecific inhibitors from the sera, according to the World Health Organization guidance on influenza diagnosis and surveillance and as previously described [17,18]. Reagents used for testing were standardized fresh red blood cells (RBC) of turkeys in Alsever's solution (Bundesinstitut für Risikobewertung, Alt-Marienfelde, Berlin, Germany) and H1N1-Virus split antigen (A/California/7/2009 NYMC X-179A; Pandemrix; GlaxoSmithKline Biologicals). Titers below the detection limit of 1:10 were assigned a value of 1:5 for the purpose of calculating the geometric mean titer. In accordance with the

European and international guidance, seroconversion after vaccination was defined by either an AB titer of $\leq 1:10$ before and $\geq 1:40$ after or $\geq 1:10$ before and ≥ 4 -fold increase in AB titer 21 days after vaccination [14,17], respectively.

Statistical Analysis

To compare antibody titers, the geometric mean with the corresponding 95% confidence interval (CI) was calculated. Other values are shown as mean values \pm standard deviation if not indicated otherwise. Continuous variables were compared using the Wilcoxon matched pairs test, nominal values were compared using Fisher's exact test. All tests were 2-tailed for a significance level of .05. Statistical analyses were done using SPSS for Windows, release 16 (SPSS Inc., Chicago, IL).

RESULTS

The study was started on November 11, 2009, and the last blood sample was taken on February 1, 2010. Seventeen patients (5 females) were included in the study and received 1 dose of the AS03-adjuvanted H1N1 influenza vaccine. All 17 patients were offered a second dose of the vaccine 21 days after the first dose. Eleven of these 17 patients received a second dose 3 weeks after the first dose and had serum samples taken on days 0, 21, and 42 available for analysis. The other 6 patients decided against a second dose, because local and federal health authorities publicized a single dose. None of the patients refused the second dose because of adverse events.

Before vaccination (day 0), 3 of 17 patients (17.6%) had a seroprotective HAI titer of 1:40 or more. Three weeks (day 21) after the first dose of the H1N1 influenza A vaccine, 9 of 17 patients (52.9%) and 3 weeks after the second dose (day 42), 10 of 11 patients (90.9%) had a seroprotective HAI titer of 1:40 or more. Seroconversion was detected in 41.2% (7 of 17 patients) at day 21 after the first and in 81.8% (9 of 11 patients) at day 42 following the second dose of the H1N1 influenza A vaccine (Table 1). When focusing on the subgroup of 11 patients who received 2 doses, 6 patients failed to seroconvert after the first dose, but 4 of these 6 seroconverted after the second dose. Accordingly, the geometric mean HAI titer increased significantly after the second dose when compared to the HAI titer developed after the first dose ($P = .002$).

The geometric mean HAI titer for patients with seroconversion after 1 dose was 182 (95% CI, 119-280) compared to 13.8 (95% CI, 5.2-36.9) for those who failed to seroconvert ($P = .003$).

The mean time between vaccination and HSCT was 19.7 (range: 4.7-49.3) months. Two patients

Table 1. Rates of Seroconversion and Seroprotection Before, After 1 and 2 Intramuscular Doses of the Novel adjuvanted H1N1 influenza vaccine (A/California/7/2009) in Patients after HSCT

		Day 0 (Before Vaccination)	Day 21 (After First Dose)	Day 42 (After Second Dose)
Geometric mean HAI titer—value (95% CI)	n = 17	7.7 (4.6-13.0)	40 (16.8-95.2)	na
	n = 11	9.7 (4.3-22)	59.6 (20.2-175)	381 (121-1195)
Seroprotection rate—% (95% CI)	n = 17	17.6 (3.8-43.4)	52.9 (27.8-77)	na
	n = 11	27.3 (6.0-61.0)	63.6 (30.8-89.1)	90.9 (58.7-99.8)
Seroconversion rate—% (95% CI)	n = 17	na	41.2 (18.4-67.1)	na
	n = 11	na	45.5 (16.8-76.6)	81.8 (48.2-97.7)

n indicates number of patients; HAI, hemagglutination inhibition test; 95% CI, 95% confidence interval; na, not applicable; HSCT, hematopoietic stem cell transplantation.

All 17 included patients were offered a second dose, which was administered to 11 of the 17 patients. Results of all patients (n = 17) and of those with a second dose (n = 11) are shown separately. Seroprotection was defined as an AB titer of $\geq 1:40$. Seroconversion was defined as an AB titer of $\leq 1:10$ before and $\geq 1:40$ after or $\geq 1:10$ before and ≥ 4 -fold increase in AB titer after vaccination.

received vaccination within 6 months after HSCT, whereupon 1 did seroconvert and 1 did not. The latter seroconverted after the second dose, which was administered 6.7 months after HSCT. Six patients had myeloablative and 11 patients reduced-intensity conditioning (RIC) before allogeneic (n = 14) and autologous HSCT (n = 3). Chronic graft-versus-host disease (cGVHD) was present in 7 of the 14 patients following allogeneic HSCT. None of the patients had acute GVHD (aGVHD) at the time of vaccination. Three patients experienced new onsets or worsening of the cGVHD after vaccination. Apart from that, the vaccine was well tolerated and no other severe clinical adverse events were found in our study.

Patients who received immunosuppressive therapy at the time of vaccination to treat or prevent GVHD after allogeneic HSCT were far more likely not to seroconvert after 1 dose of the vaccine ($P = .003$). Single or combined immunosuppressive therapy consisted of calcineurin inhibitors (n = 6), corticosteroids (n = 3), mycophenolate mofetil (n = 2), everolimus (n = 1), and rituximab within 6 months before vaccination (n = 1). However, the only 2 patients who failed to seroconvert after 2 doses of the vaccine were both <60 years old and not receiving any immunosuppressive therapy. One of them was female and received allogeneic HSCT after RIC 49.3 months before vaccination. The other patient was male and was vaccinated 25.7 months following autologous HSCT after myeloablative conditioning. In contrast to the latter described patient, the other 2 autologously transplanted patients seroconverted after the first dose of the vaccine, and AB titers hugely increased after the second dose.

No significant difference was found between the 2 groups when comparing for age, sex, type of HSCT, time elapsed after HSCT, or H1N1 prevaccination titer. Neither the CD4 cell count nor the immunoglobulin G levels differed between the 2 groups. Table 2 summarizes the major clinical and laboratory characteristics of the enrolled patients.

DISCUSSION

In our study, the rate of patients with seroprotection (HAI titer of $\geq 1:40$) increased from 17.6% to 52.9% after the first and to 90.9% after the second dose of the adjuvanted H1N1 vaccine. Likewise, the rate of seroconversion increased from 41.2% after the first to 81.8% after the second dose of the vaccine.

The European guidance for the evaluation of influenza vaccines suggests a seroconversion rate of more than 40% as well as a seroprotection rate of more than 70% after influenza vaccination of 18- to 60-year-old adults in order to be judged as efficacious [19]. After application of 1 dose, the seroconversion rate was only marginally above the recommended rate and the seroprotection rate was lower. After 2 doses, the recommended cutoffs were well exceeded.

Up to now, there has been only 1 published study, by Issa et al. [16], who measured the AB titer in 82 HSCT patients at variable time points after a single dose of a nonadjuvanted H1N1 2009 influenza A vaccine and found a seroprotection rate of 51%, which is well in agreement with our smaller study using an adjuvanted H1N1 2009 influenza A vaccine. Seroconversion rates could not be specified, because prevaccination titers were not assessed in this study. In the study by Issa et al. [16], immunosuppressive therapy was not associated with response to vaccination, whereas in our study, this was the only significant difference comparing patients who seroconverted after 1 dose of the vaccine to those who did not. Interestingly, the only 2 patients not responding after the second dose of the vaccine were both not receiving immunosuppressive therapy.

Comparing our results to earlier studies investigating the efficacy of seasonal influenza vaccines in HSCT recipients has several limitations. The most important limitation is the small size of our study. Moreover, the so-far published studies are very heterogeneous and include patients with a variety of hematologic malignancies who have received different interventions and treatments at different time points relative to the

Table 2. Demographic and Disease-Related Characteristics of the Participants Comparing Those Who Seroconverted After the First Dose to Those Who Did Not

	Seroconverter	NonSeroconverter	P Value
n (%)	7 (41.2)	10 (58.8)	
Age (years)—mean (\pm SD)	46.7 (\pm 19.4)	44.4 (\pm 11.4)	.8
Male sex—n (%)	5 (71.4)	7 (70)	1.0
Time since HSCT (months)—mean (\pm SD)	15.4 (\pm 6.7)	22.7 (\pm 17.7)	.7
Myeloablative chemotherapy before HSCT—n (%)	4 (57)	2 (20)	.2
Allogeneic HSCT—n (%)	5 (71.4)	9 (90)	.5
Chronic GVHD at the time of vaccination—n (%)	3 of 5 following allogeneic HSCT (60%)	4 of 9 following allogeneic HSCT (44%)	1.0
Any immunosuppressive therapy—n (%)	0 of 5 following allogeneic HSCT (0%)	8 of 9 following allogeneic HSCT (89%)	.003
Geometric mean HAI titer before the vaccination (day 0)—value (95% CI)	6.3 (3.6-11)	8.8 (3.6-21.4)	.5
Geometric mean HAI titer after the first dose (day 21)—value (95% CI)	182 (119-280)	13.8 (5.2-36.9)	.003
CD4 ⁺ cells (/ μ L)—mean (\pm SD)	250.7 (\pm 127.1)	349.9 (\pm 423.9)	.6
Immunoglobulin G (mg/dL)— mean (\pm SD)	768.0 (\pm 341.5)	713.9 (\pm 353.4)	.8

N indicates number of patients with antibody titers measured before and 21 days after vaccination; SD, standard deviation; HSCT, hematopoietic stem cell transplantation; 95% CI, 95% confidence interval; P values are given for the comparison of seroconverter and nonseroconverter; HAI, hemagglutination inhibition test; GVHD, graft-versus-host disease.

Most values are given as means \pm standard deviation unless otherwise stated.

vaccination studied. Immunosuppressive agents, especially monoclonal antibodies such as rituximab, can lower the ability to respond to a novel antigen, but may not limit responses to recall antigens [20]. Moreover, seasonal influenza strains can circulate over several years, and many patients are found to have high AB titers before vaccination, which is known to affect seroconversion rates [21]. Thus, the calendar year in which the influenza vaccination study was performed also needs to be taken into account. The 2009 H1N1 influenza A is a new variant with limited AB crossreactivity to earlier seasonal H1N1 influenza A strains. Therefore, investigating these new vaccines could offer new insights in a patient population with low rates of protective prevaccination immunity [22].

In accordance with the variety of influenza vaccination studies in HSCT recipients, the rate of seroconversion after 1 dose was much lower in our study compared to studies in healthy adults reporting rates of >95% [23]. Whether a second dose of an influenza vaccine can enhance immune response in patients at risk for not responding has been the subject of several studies. In healthy elderly individuals (>61 years), 3 studies (2 with seasonal and 1 with a novel H1N1 2009 vaccine) showed that a second dose of these nonadjuvanted influenza vaccines enhances rates of seroprotection and seroconversion [24-26]. Two studies in younger individuals (<12 years), 1 using a nonadjuvanted and 11 an adjuvanted H1N1 influenza A vaccine, showed increased seroprotection rates after a second dose [27,28]. A previous report from our group showed a significantly increased rate of seroconversion in HIV-1 infected patients after 2 doses of the novel adjuvanted H1N1 vaccine [29]. In patients with malignancies, 2 smaller, nonrandomized studies found an increased seroconversion rate [30,31], but a third nonrandomized study in HSCT recipients [32], as well as 1 small, ran-

domized trial, failed to show a difference after 2 doses of a nonadjuvanted seasonal influenza vaccine [33].

In our study, a second dose of the adjuvanted H1N1 vaccine resulted in a significant increase of protective AB titers. Most studies in HSCT patients, including ours, are small and lack randomization; thus, clear answers to the question of the time point of vaccination and the clinical benefit of a second dose do not exist. But this issue needs to be addressed. Seasonal influenza, as well as 2009 H1N1 influenza A, is a relevant threat to HSCT recipients, with a reported case fatality ranging up to 33%.

The 2009 H1N1 vaccine was overall well tolerated in our small cohort. Both, Issa et al. [16] (n = 82) using a nonadjuvanted, and Ditschkowski et al. [11] (n = 55) using an adjuvanted vaccine, described the H1N1 vaccine as well tolerated and safe.

In summary, our results suggest that 1 dose of the split virion, inactivated, adjuvanted pandemic H1N1 influenza A vaccine resulted in a low rate of protection in patients with hematologic malignancies after HSCT. A second dose seems to enhance protection and should be systematically studied.

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AUTHORSHIP STATEMENT

R.A. established and measured the HAI. E.H. did the statistical analyses. All other authors were involved in writing the protocol and manuscript, and consented, treated, and vaccinated the patients.

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