

Original Article

Immunogenicity and Safety to Influenza A [H1N1] Vaccine in Hematologic Disease Patients on Immunosuppressive Therapy

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Abstract: Recent studies have demonstrated low immunogenic response to influenza vaccine among hematologic malignancy patients who are treated with chemotherapy or are undergoing stem cell transplantation. There is limited data on response to the new Influenza A H1N1 vaccine on hematologic malignancy patients and no data on benign hematologic patients who received immunosuppressive therapy. This study aims to determine the efficacy and safety of Influenza A H1N1 vaccine on benign hematologic patients who on immunosuppressive therapy and hematologic malignancy patients who received chemotherapy. **Materials and Methods:** The study population comprised 15 hematologic patients and 60 healthy control subjects who underwent split-virion, inactivated monovalent Influenza A [H1N1] vaccination. Sera were obtained before, four weeks after, and twenty-four weeks after vaccination. Haemagglutination inhibition assays were used to evaluate the antibody responses. **Results:** The mean age of the enrolled population was 46.2 years for hematologic disease patients and 42.5 years for control subjects [$p = 0.29$]. The seroprotection rate at four weeks was 46.7% in hematologic disease patients and 66.7% in healthy control subjects [$p = 0.152$]; by twenty-four weeks, the seroprotection rate was 18.2% in hematologic disease patients and 42.4% in healthy control subjects [$p = 0.13$]. The seroconversion rate was 46.7% in hematologic disease patients and 61.7% in healthy controls at four weeks [$p = 0.29$]; by twenty-four weeks, the seroconversion rate was 18.2% in hematologic disease patients and 30.5% in healthy controls [$p = 0.40$]. **Conclusions:** The influenza A [H1N1] vaccine in hematologic disease patients is safe and effective.

Key Words : ● Immunogenicity ● Safety ● Influenza A [H1N1] vaccine

● Hematologic disease ● Immunosuppressive therapy

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Introduction

In March 2009, a series of severe influenza cases were described among otherwise healthy Mexican young adults¹ and within weeks, thousands of late season influenza cases were reported throughout the world.² The 2009 H1N1 pandemic infection rates have

been highest among persons less than 25 years of age, but death rates have been highest among persons 25-49. One potential explanation for these trends is that exposure to strains of influenza may confer some protective neutralizing antibody titer against 2009 H1N1.³⁻⁵ The higher prevalence of co-morbidities and immune impairment lead to higher morbidity and mortality rates among persons who become infected.

Patients with hematologic malignancies are likely to be at an increased risk for infection with influenza. A

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devastating complication of influenza infection is lower respiratory tract disease and pneumonia, complicated by bacterial or fungal co-infection, frequently leading to acute lung injury and death. Most patients with influenza infection and hematologic malignancies present with symptomatic upper respiratory symptoms. However systemic symptoms such as fever, myalgia and fatigue may be reduced or be completely absent, especially in the hematologic stem cell transplantation population. The use of corticosteroids may play an additional role in these patients. A meta-analysis showed that the mortality rate in hematologic malignancy and hematopoietic stem cell transplantation patients from influenza infection is about 17% (0-33%).⁶ Vaccination for individuals who are at increased risk for influenza infection and influenza-associated pneumonia has been recommended by the Centers for Disease Control and Prevention. This would include the elderly, individuals who have chronic disease or a malignancy, and those who are currently receiving immunosuppressive medications (e.g., corticosteroids, chemotherapy).⁷⁻⁹ Although numerous studies have shown that the inability of the individuals to mount an adequate immunologic response to a variety of vaccines depends both on the underlying hematologic malignancy and its therapy.^{6,10-13,21} Patients immediately preceding or in the 6 months following myeloablative conditioning for hematologic stem cell transplant¹⁴, or those who are within seven days after receipt of conventional chemotherapy are unlikely to mount a protective response^{15,16} and should have vaccination deferred; but all others are recommended to receive both seasonal and 2009 H1N1 vaccines and should preferentially receive inactivated influenza vaccines.

The existing data on vaccine efficacy in hematologic malignancy patients using pre- and post-immunization serum antibody titers with a 4-fold increase in antibody titer or $\geq 1:40$ as indicative of immune responses vary from 10-90%^{10-13, 21-23} with a low of 19% in adults with multiple myeloma to about 50% in lymphoma patients.^{10-13, 16,20} The impairment of normal B cells function from natural

history of disease may effect on antibody production in response to vaccine. The data of other hematologic disease patients who are on immunosuppressive drugs such as steroid also limited. Experimental studies have shown a pro-apoptotic effect of dexamethasone on T lymphocytes³² suggesting that glucocorticoids may direct T-cell positive and negative selection in the thymus, limit activation-induced cell death during the contraction phase of an adaptive immune response and induce generalized thymocyte apoptosis after polyclonal T-cell activation.³³ Moreover, steroid causes a reduction of splenic and lymph node B-cell numbers and attenuation of early B-cell progenitor proliferation.³⁴ These effects on metabolism and distribution of T and B lymphocytes may reduce effectiveness of vaccine.

In this study, we describe the humoral immune response including the seroprotection, seroconversion and seroresponse rates in A/H1N1 influenza vaccinated hematologic patients under immunosuppressive therapy and also confirm the safety of this A/H1N1 vaccine.

Materials and Methods

Fifteen Thai patients with a diagnosis of hematologic disease (6 Autoimmune hemolytic anemia, 4 Idiopathic thrombocytopenic purpura, 4 non-Hodgkin's lymphoma (3 Diffuse large B cell lymphoma, 1 MALT lymphoma, 1 multiple myeloma) under immunosuppressive agents such as steroid or active chemo/immunotherapy at enrollment or completed within the last 3 months were recruited from the Panyanantaphikkhu Chonprathan Medical Center and Ongkaruk, Srinakarinwiroj University. Subjects were required to be at least 15 years of age. The mean age of the enrolled population was 46.2 years for hematologic disease patients and 42.5 years for controls ($p = 0.29$) with age range of 26-62 years for cases group and 26-59 years for controls. Subjects were excluded from eligibility into the study if they had a history of the following: (1) known or suspected allergy to eggs or egg products, thimerosal and gentamicin (2) history of life-threatening reaction to prior influenza vaccination (3)

received immunoglobulin within the last three months prior to vaccination (4) thrombocytopenia or bleeding disorder contraindicating intramuscular injection (5) pregnancy (6) laboratory-confirmed infection with H1N1 (2009) (7) blood component transfusion within the last three months.

The controls were sex- and age- matched healthy volunteers recruited from Panyanantaphikkhu Chonprathan Medical Center personnel. The ratio of cases and controls was 1:4 with statistical significant at 0.05 and the statistical power 80%. The study was approved by the ethical committee before enrollments.

A single dose of 0.5 mL of vaccine contained 15 µg of monovalent split swine A/H1N1 influenza vaccine, without the adjuvant (Sanofi Pasteur, Lot no.E5937-7132), was administered intramuscularly. Antibody titers to the antigen contained in the vaccine were measured prior to administration and again at 4 and 24 weeks subsequent to vaccination by means of the hemagglutinin inhibition test. All subjects were questioned about the occurrence of clinical systemic symptoms and/or local adverse effects around the injection site including experience of fever, chills, malaise, myalgia, headache, pain, swelling, rash, edema, nausea, and neurological symptom within 7 days after vaccination and throughout 6 months of participation to identify possible local and systemic adverse reactions.

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) 16 software. The difference in age between patient groups and control groups was tested using independent t-test to compare means. The seroconversion, seroprotection and seroresponse rate were compared between groups using chi square test. A p value < 0.05 was considered significant.

Results

Demographics

Fifteen Thai hematologic patients and sixty normal controls were enrolled into this study. The demographic features of the hematologic patients and normal controls are shown in Table 1. The mean age of the enrolled population was 46.2 years for hematologic disease patients and 42.5 years for control subjects (p = 0.29). Most are female (86.7% in hematologic patients and 91.7% in controls). Their body weights ranged from 45-80 kilograms, and all of them are non-smokers. Of those, 66.7% of patients had benign hematologic disease taking prednisolone, average dose of 12 milligram per day, and 33.3% had hematologic malignancy receiving chemotherapy (CVP: Cyclophosphamide, Vincristine, Prednisolone; CHOP: Cyclophosphamide, Vincristine, Doxorubicin, Prednisolone; R-ESHAP: Rituximab, Etoposide, Methylprednisolone, Cisplatin, Cytarabine, and MPT:

Table 1 Characteristic of patients and controls

	Patients	Controls	p value
Number	15	60	
Age (mean)	46.26	42.55	0.29
Sex (male : female)	1 : 6.5	1 : 11	0.55
Seroprotection rate before vaccination	6.67% (n = 1)	16.67% (n = 10)	0.39
Type of disease (benign : malignancy)	66.7 : 33.3		
Diagnosis			
● ITP	4 (26.7%)		
● AIHA	6 (40.0%)		
● NHL	4 (26.7%)		
● MM	1 (6.7%)		

Melphalan, Prednisolone, Thalidomide). Seven percent of cases and 15% of healthy controls had seroprotection before vaccination ($p = 0.39$)

At 4 weeks, seroprotection rate increased significantly, compared with those before vaccination (Figure 1 and 2). The seroprotection rate at 4 weeks was 46.7% in hematologic disease patients and 66.7% in healthy control subjects ($p = 0.152$); by 24 weeks, the seroprotection rate was 18.2% in hematologic disease patients and 42.4% in healthy control subjects ($p = 0.13$). The seroconversion rate was 46.7% in hematologic disease patients and 61.7% in healthy controls at 4 weeks ($p = 0.29$); by 24 weeks, the seroconversion rate was 18.2% in hematologic disease patients and 30.5% in healthy controls ($p = 0.40$). The seroresponse rate at 4 weeks was 46.7% in hematologic disease patients and 61.7% in healthy control subjects ($p = 0.29$); by 24 weeks, the seroresponse rate was 18.2% in hematologic disease patients and 30.5% in healthy control subjects ($p = 0.40$). Twenty-seven percent of patients lost follow up at 24 weeks while there was 1.7% loss in controls.

The seroprotective, seroconversion and sero-response rates of antibodies toward the antigens did not significantly

differ between patients and controls, at both 4 weeks and 24 weeks. The seroprotective, seroconversion and seroresponse rates were lower at 24 weeks than at 4 weeks in both hematologic patients and normal controls. However, there is no statistical significance between two groups. (Table 2)

We also analysed the seroprotection, seroconversion and seroresponse rates at 4 weeks and 24 weeks after vaccination in each type of disease, benign hematologic disease and hematologic malignancy, with the controls. No difference was found in the percentage of seroprotection, seroconversion and seroresponse rates at 4 weeks and 24 weeks in both benign hematologic disease and hematologic malignancy group. Twenty percent of patients in the benign hematologic disease group and 40% of patients in hematologic malignancy group lost follow up at 24 weeks while there was 1.7% loss in controls.

Safety

No significant difference in systemic adverse reactions between hematologic patients and healthy controls except for nausea, is found significantly more in hematologic patients than healthy controls (Table 3)

Table 2 The percentage of seroconversion, seroconversion and seroresponse rate in hematologic disease patients and controls

Percentage	4 weeks			24 weeks		
	Patient (n = 15)	Control (n = 60)	p value	Patient (n = 11)	Control (n = 59)	p value
Seroprotection	46.7%	66.7%	0.15	18.2%	42.4%	0.13
Seroconversion	46.7%	61.7%	0.29	18.2%	30.5%	0.40
Seroresponse	46.7%	61.7%	0.29	18.2%	30.5%	0.40

Table 3 Adverse reaction in hematologic disease patients and controls

	Patients (%)	Controls (%)	p value
Fever	16.7	7.1	0.41
Pain	18.2	26.8	0.54
Swelling	0	1.8	0.65
Redness	0	7.1	0.36
Ecchymosis	0	1.8	0.65
Headache	30.0	12.5	0.15
Myalgia	40.0	19.6	0.15
Malaise	30.0	10.7	0.10
Rash	0	1.8	0.67
Nausea	30.0	7.1	0.03
Edema	0	NA	NA
Dizziness	10.0	NA	NA

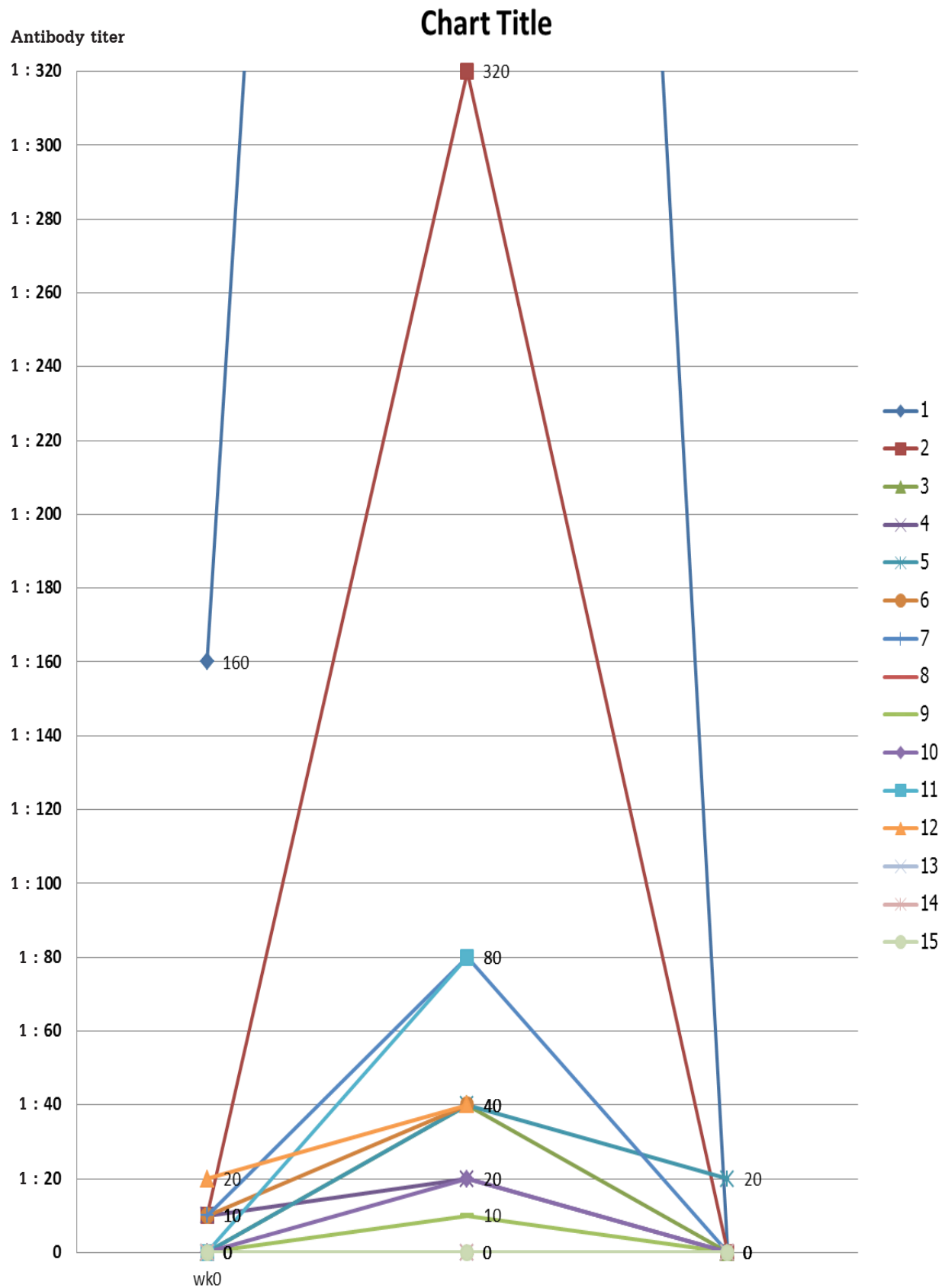


Figure 1 HAI titer of patients at week 0, 4, 24 (Extended)

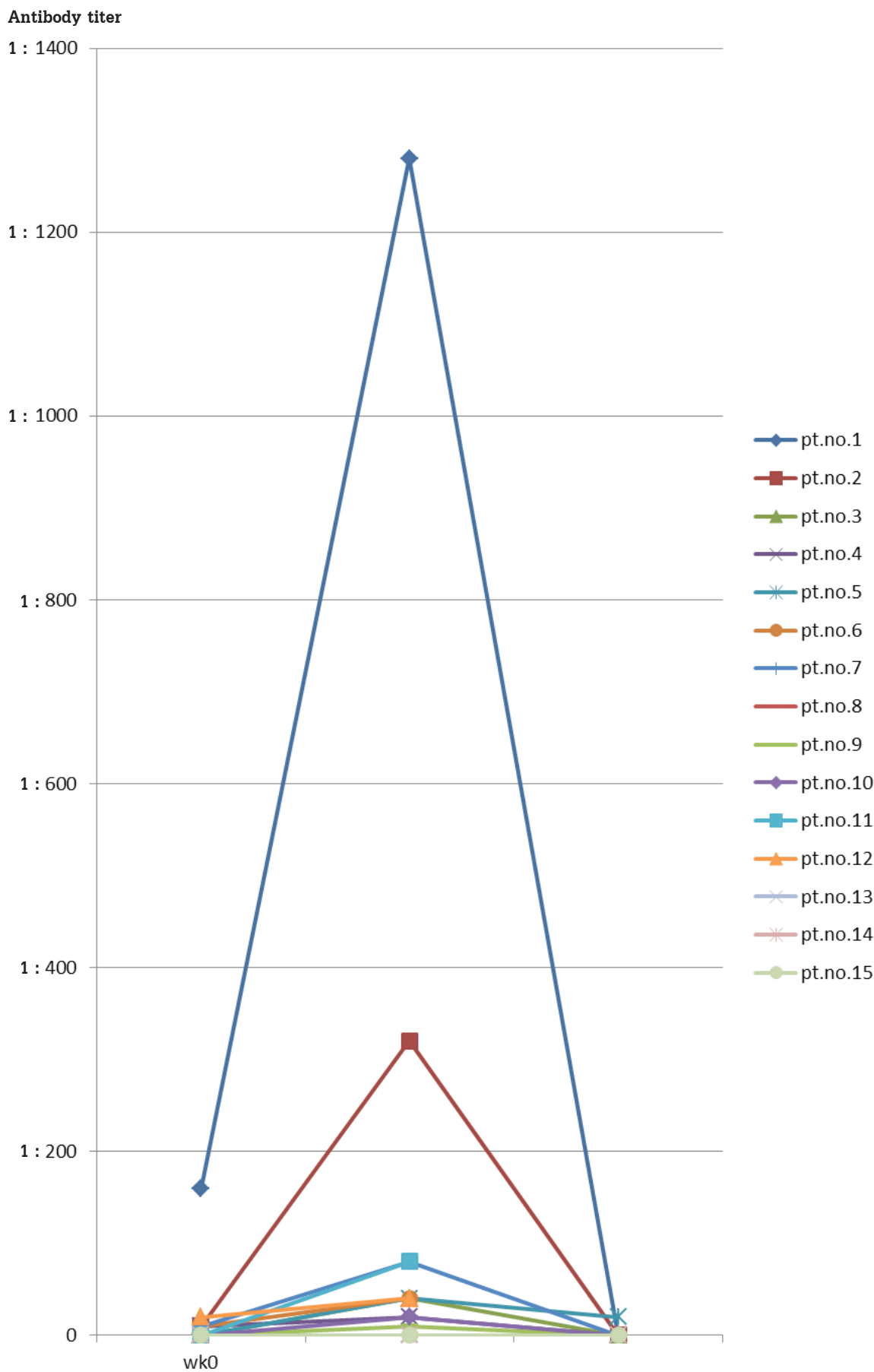


Figure 2 HAI titer of patients at week 0, 4, 24

Discussion

The influenza A (H1N1) vaccine is safe and effective in hematologic disease patients and has no obvious adverse clinical effects. But patients tend to reduce humoral and cell-mediated immunity, such as a more rapid decline in anti-influenza antibody titers. In our study, 6.7% of patients and 15% of controls had protective antibodies against H1N1 at the time the pre-vaccination samples were collected, perhaps due to exposure to the H1N1 virus prior to the time of sample collection or exposure to a similar strain of virus during a previous flu season. However, there was no statistical difference between those two groups.

No difference was found in percentage of seroprotection, seroconversion and seroresponse rates at 4 weeks in benign hematologic disease group may cause by less power effect.

A trend toward a lower humoral response to influenza vaccination in patients taking prednisolone due to effects of corticosteroids on lymphocyte populations involved in immunoglobulin biosynthesis. B cell responsiveness is diminished and suppressor T lymphocyte activity is removed²⁴⁻²⁸, although our reports showed the response did not differ between patients with benign hematologic disease, treated with prednisolone, and healthy controls. These results are the same as other studies in rheumatic diseases, treated with immunosuppressive therapy, including systemic lupus erythematosus (SLE) disease, the chronic inflammatory disease with defect in B lymphocytes which causes abnormal production of autoantibodies and has the same pathogenesis as benign hematologic disease in this study (AIHA, ITP). Those results showed no significant differences in the increase of titers to influenza vaccine between rheumatic patients and controls^{29,31}. A trial in rheumatic disease reported less mean increase of

titer to influenza vaccine in young patients treated with glucocorticoids³⁰. Even though our study is quite small and some data are missing in prednisolone dosage and duration, our study is the first study that looks at benign hematologic disease in those receiving prednisolone. More and larger studies are needed to clarify this effect of prednisolone dosage and duration to the response of influenza vaccine.

Immune response to the influenza H1N1 vaccine in hematologic malignancy patients in this study was lower than the rate previously reported in the previous influenza vaccine studies¹⁰⁻¹³, including one that studied on H1N1 2009-2010 vaccine¹⁰. This may due to both the effects of the malignancies and the chemotherapeutic agents used in treatment which may have impaired the ability to generate a protective immune response to vaccination. A difference between the vaccine used in this study and that used in previous H1N1 2009-2010 study¹⁰ is that we used unadjuvanted vaccines while previous trials used an oil-in-water adjuvant that enhanced the immune response to vaccines by triggering the release of chemokines and stimulating the maturation of dendritic cells and monocytes.

However, these results are similar to multiple prior studies in hematologic malignancy patients using unadjuvanted vaccines that report the influenza vaccine did induce a measurable antibody titer, but at a lower rate than that observed in healthy people¹¹⁻¹³. Two studies have shown that a second dose of influenza vaccine can enhance the antibody response in the patients receiving chemotherapy who mounted an inadequate immunologic response to the initial dose^{18,19}. Large multicenter studies will be necessary to obtain accurate data about the efficacy of influenza A (H1N1) vaccine and the need for second shot.

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การศึกษาการตอบสนอง และความปลอดภัยต่อการได้รับวัคซีนไข้หวัดใหญ่ สายพันธุ์ใหม่ชนิดเอ (เอช 1 เอ็น 1) ในผู้ป่วยโรคทางโลหิตวิทยาที่ได้รับ ยากดภูมิคุ้มกัน

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บทคัดย่อ ผู้ป่วยโรคกระดูกพรุนทางโลหิตวิทยาที่ได้รับยาเคมีบำบัด มีการตอบสนองของภูมิคุ้มกันต่อวัคซีนไข้หวัดใหญ่ในระดับต่ำ และข้อมูลการตอบสนองของภูมิคุ้มกันต่อวัคซีนไข้หวัดใหญ่สายพันธุ์ใหม่ ชนิดเอ (เอช 1 เอ็น 1) ในผู้ป่วยกลุ่มนี้ยังมีอยู่จำกัด และไม่เคยมีการศึกษาในโรคทางโลหิตวิทยาอื่นๆ ที่ได้รับยากดภูมิคุ้มกัน งานวิจัยนี้จึงมีวัตถุประสงค์เพื่อศึกษาประสิทธิภาพและความปลอดภัยของวัคซีนไข้หวัดใหญ่สายพันธุ์ใหม่ชนิดเอ (เอช 1 เอ็น 1) ในผู้ป่วยโรคกระดูกพรุนทางโลหิตวิทยาที่ได้รับยาเคมีบำบัด และโรคทางโลหิตวิทยาอื่นๆ ที่ได้รับยากดภูมิคุ้มกัน **วัสดุและวิธีการ** ผู้ป่วยโรคทางโลหิตวิทยา 15 คน และอาสาสมัคร 60 คน ได้รับวัคซีนไข้หวัดใหญ่สายพันธุ์ใหม่ชนิดเอ (เอช 1 เอ็น 1) และได้รับการเก็บน้ำเหลืองในช่วงก่อนฉีดวัคซีน หลังฉีด 4 สัปดาห์ และ 24 สัปดาห์ เพื่อตรวจหาภูมิคุ้มกัน โดยวิธี haemagglutination inhibition assay **ผลการศึกษา** ผู้ป่วยโรคเลือดมีอัตราการเกิด seroprotection ร้อยละ 46.7 เทียบกับ ร้อยละ 66.7 ในอาสาสมัครกลุ่มควบคุม ที่ 4 สัปดาห์ ($p = 0.152$) และ ร้อยละ 18.2 เทียบกับ ร้อยละ 42.4 ที่ 24 สัปดาห์ ($p = 0.13$) และอัตราการเกิด seroconversion ร้อยละ 46.7 เทียบกับ ร้อยละ 61.7 ที่ 4 สัปดาห์ ($p = 0.29$) และ ร้อยละ 18.2 เทียบกับ ร้อยละ 30.5 ที่ 24 สัปดาห์ ($p = 0.40$) โดยทั้งสองกลุ่มมีผลข้างเคียงไม่แตกต่างกัน **สรุป** วัคซีนไข้หวัดใหญ่สายพันธุ์ใหม่ชนิดเอ (เอช 1 เอ็น 1) มีประสิทธิภาพในผู้ป่วยโรคทางโลหิตวิทยา

Key Words : ● Immunogenicity ● Safety ● Influenza A [H1N1] vaccine
● Hematologic disease ● Immunosuppressive therapy

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2556;23:43-51.

