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RESEARCH PAPER

Immune response to influenza vaccination in children with cancer

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ABSTRACT

The aim of this study was to evaluate the ability of influenza immunization to evoke a protective immune response among children with cancer. We evaluated 75 children with cancer who received influenza vaccination. Hemagglutination Inhibition Antibody titers were determined before and after vaccination. The protective rates after vaccination were 79% for H1N1, 75% for H3N2 and 59% for influenza B virus whereas the seroconversion rates were 54%, 44% and 43% respectively. The differences pre- and post-vaccination were significant regardless the method which was used: seroprotection changes, seroconversion and geometric mean titers analyses. Variables such as the pre-vaccination antibody titers, the time when the responses were measured after the vaccination, the age and the type of malignancy as well as the absolute lymphocyte count were found to be correlated with the immune response but the findings were different for each vaccine subunit. In conclusion, influenza vaccination provides protection in a remarkable proportion of pediatric cancer patients whereas this protection is more obvious against H1N1 and H3N2 compared to influenza B. The immune response after vaccination is significant and seems to be influenced by a variety of factors.

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Introduction

Influenza virus usually runs a benign course even among children with cancer. Nevertheless, children with malignancies are at increased risk of contracting influenza and experiencing prolonged infection or severe influenza related complications including intensive care admissions and higher mortality rates.[1-4] Moreover, in this group of children, influenza infection may cause delays or an interruption of chemotherapy and consequently longer hospitalization periods.[2,3] For the above mentioned reasons clinicians and public health authorities recommend annual vaccination against influenza especially for this population.[5] Influenza vaccines have shown to be safe and effective in healthy children, but for children with cancer who may have a decreased response because they are immunocompromised due to their disease and the immunosuppressive treatment they receive, the published data are limited and conflicting.[6-8]

In the past, some studies have reported an adequate antibody response to influenza vaccine among immunized children with cancer, although delayed or lower than among healthy individuals.[8-11] To date, no major concerns regarding the safety of influenza vaccines have been reported.[7,8,12] Nevertheless, concerns regarding the effectiveness of influenza immunization in children with cancer may explain the low compliance rate in this population.[13,14] However, we have previously reported high compliance rates from our center over the 2009–2012 influenza seasons with an increasing rate from 87 to 95%.[15] In this new study, from our department, our aim was to evaluate the ability of trivalent inactivated influenza vaccine to evoke a protective immune response among children with cancer.

Results

Immunization against influenza was conducted in a total of 107 patients. Twelve children had been excluded because they did not fulfill the criteria established for the vaccination. Five families refused the vaccination (compliance rate: 107/112 – 95.5%).

Sera before vaccination were taken from 97 children whereas paired sera (before and after vaccination) were obtained in 75 children. Consequently, 75 pediatric patients with cancer (38 males and 37 females) with a median age of 8.8 years (range 1.4 – 16.9) were finally enrolled. Second blood sample was collected within or after 45 days after the final dose of vaccine in 35 and 40 patients respectively. In only 5 patients the time between the final vaccination dose and collection of second sample was more than 90 days. No laboratory proven influenza infection was recorded among immunized children. Table 1 shows the characteristics of children finally included in the study. No differences were detected among patients with a second blood sample

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Table 1. Patients' characteristics (n = 75).

Characteristic	Total
Gender	
Male	38 (50.7)
Female	37 (49.3)
Age (years)	
<3	2 (2.7)
3 – 9	38 (48)
> 9	35 (49.3)
Cancer type	
Leukemia	48 (64)
Lymphoma	5 (6.7)
Solid tumors	22 (29.3)
Dosing schedule	
1 dose	51 (68)
2 doses	24 (32)
Treatment	
On	62 (82.7)
Off	13 (17.3)
Treatment Intensity	
Intensive	29 (46.8)
Less Intensive	33 (53.2)
Time since the last treatment days / median (range)	3 (0–180)
WBC* (/mm^3) median (range)	4100 (1200–23000)
Neutrophils* (/mm^3) median (range)	2126 (546–11040)
Lymphocytes* (/mm^3) median (range)	1238 (156–3947)
≤1000	21 (29.0)
>1000	51 (71.0)
Sampling time since the last dose days / median (range)	48 (28–173)
≤45 days	35 (46.7)
>45 days	40 (53.3)

Data are number (percentage) of patients, *at the time of vaccination, for 3 patients, the count of lymphocytes was missing.

collected within 45 days after the vaccination and those with a second sample collected after 45 days regarding their demographic characteristics.

Safety evaluation

The vaccine was found to be well tolerated in all children. Only mild local vaccine related reactions were reported: erythema, swelling, mild pain or combinations in 4 children as well as low grade fever within the next 48 hours after the vaccination in 2.

Seroprotection rate analysis

Findings are shown in the Table 2. Fourteen patients (19%) were susceptible to all three influenza viruses and 11 (15%) showed protective titers against all three viruses prior to vaccination. After vaccination, 36 patients (48%) showed protective titers against all three viruses whereas only 6 (8%) were susceptible to all strains. The differences between pre- and post-vaccination seroprotective rates were significant for all vaccine strains in the whole cohort and regardless of the sampling time (except for Influenza B in the >45 subgroup).

Determinants of seroprotection

Findings are shown in Table 3. Most patients achieved significantly higher post-vaccination seroprotective titers, with the exception of some patient groups which failed to achieve significantly higher post-vaccination seroprotective rates: children off treatment or with ALC ≤1000/mm3 for all strains, children receiving two vaccine doses for H3N2 & B as well as females or those with a second sample collected >45 days after vaccination for the B strain. When focusing on post-vaccination seroprotective titer, factors such as ALC >1000/mm3 for H1N1 and age >9 years or solid tumors for H3N2 and B strains, were found to be correlated with a higher post-vaccination seroprotective titer.

Seroconversion rate analysis

Findings are shown in Table 2. A fourfold increase in HAI titers (seroconversion) occurred in 54% of patients for H1N1, 44% for H3N2, 43% for B strain and was found to be even higher in

Table 2. Immune response to vaccine strains.

		Total group	≤45*	>45*	(≤45 Vs >45) p=
H1N1	pre HAI titer ≥40	39/75 (52.0)	19/35 (54.3)	20/40 (50.0)	ns
	post HAI titer ≥40	59/75 (78.7)	31/35 (88.6)	28/40 (70.0)	ns
	(pre Vs post) p value	**<0.001**	**<0.001**	**0.008**	
	≥4fold increase	38/70 (54.3)	21/31 (67.7)	17/39 (43.6)	ns
	GMT pre-vaccination (95% CI)#	30.6 (22.3–41.9)	32.2 (19.4–53.4)	29.3 (19.4–44.3)	ns
	GMT post-vaccination (95% CI)#	111.6 (74.1–168.0)	169.8 (93.9–307.2)	77.3 (44.0–135.7)	ns
	(pre Vs post) p value	**<0.001**	**<0.001**	**<0.001**	
H3N2	pre HAI titer ≥40	37/75 (49.4)	16/35 (45.7)	21/40(52.5)	ns
	post HAI titer ≥40	56/75 (74.7)	26/35 (74.3)	30/40 (75.0)	ns
	(pre Vs post) p value	**<0.001**	**0.002**	**0.004**	
	≥4fold increase	29/66 (43.9)	16/31 (51.6)	13/35 (37.1)	ns
	GMT pre-vaccination (95% CI)#	34.5 (24.1–49.4)	29.1 (16.4–51.7)	40.0 (25.0–63.9)	ns
	GMT post-vaccination (95% CI)#	99.9 (66.5–149.9)	103.5 (52.8–203.0)	96.8 (58.0–161.5)	ns
	(pre Vs post) p value	**<0.001**	**<0.001**	**<0.001**	
B	pre HAI titer ≥40	29/75 (38.7)	11/35 (31.4)	18/40 (45.0)	ns
	post HAI titer ≥40	44/75 (58.7)	21/35 (60.0)	23/40 (57.5)	ns
	(pre Vs post) p value	**<0.001**	**0.002**	ns	
	≥4fold increase	29/68 (42.6)	16/33 (48.5)	13/35 (37.1)	ns
	GMT pre- vaccination (95% CI)#	23.0 (16.1–32.9)	18.1 (11.4–28.7)	28.3 (16.3–49.0)	ns
	GMT post-vaccination (95% CI)#	62.3 (39.4–98.6)	59.4 (31.9–110.8)	65.0 (32.6–129.6)	ns
	(pre Vs post) p value	**<0.001**	**<0.001**	**<0.001**	

Data are number (percentage) of patients, *time interval between final vaccination and collection of second sample, #Data are antibody titers transformed into log2, CI: Confidence Interval, ns: non significant, HAI, Hemmaglutination inhibition.

Table 3. Univariate analysis of seroprotection rates by patients' characteristics for H1N1, H3N2, B strains.

	Patients with seroprotection before and after vaccination								
	H1N1			H3N2			B		
Factor	Before: n (%)	After: n (%)	Post vs Pre, p=	Before: n (%)	After: n (%)	Post vs Pre, p=	Before: n (%)	After: n (%)	Post vs Pre, p=
Age (years)									
≤9	22/40 (55.0)	30/40 (75.0)	**0.008**	18/40 (45.0)	25/40 (62.5)	**0.016**	10/40 (25.0)	18/40 (45.0)	**0.008**
>9	17/35 (48.6)	29/35 (82.9)	<**0.001**	19/35 (54.3)	31/35 (88.6)	<**0.001**	19/35 (54.3)	26/35 (74.3)	**0.016**
(≤9 Vs 9) p=	ns	ns		ns	**0.0155**		**0.0168**	**0.0181**	
Gender									
Male	21/38 (55.3)	33/38 (86.8)	<**0.001**	17/38 (44.7)	30/38 (78.9)	<**0.001**	14/38 (36.8)	24/38 (63.2)	**0.002**
Female	18/37 (48.6)	26/37 (70.3)	**0.008**	20/37 (54.1)	26/37 (70.3)	**0.031**	15/37 (40.5)	20/37 (54.1)	ns
(Male Vs Female) p=	ns	ns		ns	ns		ns	ns	
Cancer type									
Hematological (Hem)	26/53 (49.1)	39/53 (73.6)	<**0.001**	22/53 (41.5)	35/53 (66.0)	<**0.001**	16/53 (30.2)	25/53 (47.2)	**0.004**
Solid	13/22 (59.1)	20/22 (90.9)	**0.016**	15/22 (68.2)	21/22 (95.5)	**0.031**	13/22 (59.1)	19/22 (86.4)	**0.031**
(Hem Vs Solid) p=	ns	ns		**0.0445**	**0.0079**		**0.0357**	**0.0019**	
Treatment									
On	34/62 (54.8)	50/62 (80.6)	<**0.001**	32/62 (51.6)	47/62 (75.8)	<**0.001**	23/62 (37.1)	36/62 (58.1)	<**0.001**
Off	5/13 (38.5)	9/13 (69.2)	ns	5/13 (38.5)	9/13 (69.2)	ns	6/13 (46.2)	8/13 (61.5)	ns
(On Vs Off) p=	ns	ns		ns	ns		ns	ns	
Type of Treatment									
Intensive (Int)	17/29 (58.6)	24/29 (82.8)	**0.016**	16/29 (55.2)	25/29 (86.2)	**0.004**	14/29 (48.3)	20/29 (69.0)	**0.031**
Less intensive	17/33 (51.5)	26/33 (78.8)	**0.004**	16/33 (48.5)	22/33 (66.7)	**0.031**	9/33 (27.3)	16/33 (48.5)	**0.016**
(Int Vs Less Int) p=	ns	ns		ns	ns		ns	ns	
Doses									
One	26/51 (51.0)	38/51 (74.5)	<**0.001**	26/51 (51.0)	41/51 (80.4)	<**0.001**	20/51 (39.2)	32/51 (62.7)	<**0.001**
Two	13/24 (54.2)	21/24 (87.5)	**0.008**	11/24 (45.8)	15/24 (62.5)	ns	9/24 (37.5)	12/24 (50.0)	ns
(One Vs Two) p=	ns	ns		ns	ns		ns	ns	
Lymphocytes (/mm^3)*									
≤1000	11/21 (52.4)	13/21 (61.9)	ns	12/21 (57.1)	16/21 (76.2)	ns	8/21 (38.1)	11/21 (52.4)	ns
>1000	28/51 (54.9)	44/51 (88.2)	<**0.001**	25/51 (49.0)	38/51 (74.5)	<**0.001**	19/51 (37.3)	31/51 (60.8)	<**0.001**
(≤1000 Vs >1000) p=	ns	**0.0480**		ns	ns		ns	ns	
Sampling Time									
≤45**	19/35 (54.3)	31/35 (88.6)	<**0.001**	16/35 (45.7)	26/35 (74.3)	**0.002**	11/35 (31.4)	21/35 (60.0)	**0.002**
>45**	20/40 (50.0)	28/40 (70.0)	**0.008**	21/40 (52.5)	30/40 (75.0)	**0.004**	18/40 (45.0)	23/40 (57.5)	ns
(≤45 Vs >45) p=	ns	ns		ns	ns		ns	ns	

Data are number (percentage) of patients,ns: non significant, *at the time of vaccination, **time between final vaccination and collection of second sample,for 3 patients, lymphocytes count was missing.

the group of patients with a second blood sample collected within ≤45 days vs >45 days after vaccination. Nevertheless, the differences between these two groups did not reach significance. We detected that factors such as solid tumors and pre-vaccination HAI≥40 were correlated with significantly higher seroconversion rate. Data are shown in Table 4.

Geometric mean titer analysis (GMT)

A significant increase in the GMTs after vaccination for all 3 strains was revealed. The results of comparing pre- and post-vaccine GMTs for each of the three antigens are depicted in Table 2. The same significant results were found for all strains comparing pre- and post-vaccine GMT values regardless the sampling time.

In univariate analysis, variables which were found to be correlated significantly with higher post-vaccination GMTs were GMTs before vaccination ($p<0.001$), high ALC at the vaccination time ($p = 0.015$) and solid tumors ($p = 0.042$) for H1N1. GMTs before vaccination and solid tumors were also significant factors for higher post-vaccination GMTs for both H3N2 ($p<0.001$, $p<0.001$ respectively) and Influenza B ($p<0.001$, $p<0.001$ respectively).

A multivariate analysis was also conducted concerning the response to vaccination in terms of high post-vaccination GMTs. Post vaccination antibody titers transformed into log2 were considered as dependent variable. Table 5 illustrates the multiple linear regression analysis and shows the characteristics found to be independent factors for higher post-vaccination titers.

Discussion

Our findings indicate that influenza vaccination elicits an adequate immune response in a remarkable proportion of pediatric cancer patients. In line with previous reports, influenza vaccine was shown to be well tolerated with a low reactogenicity rate.[7,8,12,16] However, immunogenicity results are not comparable in all previous studies.[6,9] Patients recruited in different studies differ with regard to cancer type and treatment status. These are probably some of the reasons that account for the variation that has been observed in seroconversion and seroprotection rates.[11]

Furthermore, there is a variety concerning the type of vaccine that has been administered and the dosage or the number of doses used.[7,8,10-12,16-27] The related studies were also conducted during different influenza seasons. To our knowledge, four studies have evaluated the use of trivalent inactivated vaccine in patients with various types of cancer as in our study.[7,8,12,23]

Table 4. Univariate analysis of seroconversion rates by patients' characteristics.

	Proportion of patients with seroconversion: n (%)		
Factor	H1N1	H3N2	B
Age (years)			
≤9	21/35 (60.0)	12/33 (36.4)	14/37 (37.8)
>9	17/35 (48.6)	17/33 (51.5)	15/31 (48.4)
p	ns	ns	ns
Gender			
Male	21/36 (58.3)	17/35 (48.6)	17/34 (50.0)
Female	17/34 (50.0)	12/31 (38.7)	12/34 (35.3)
p	ns	ns	ns
Cancer type			
Hematological	24/50 (48.0)	19/51 (37.3)	15/49 (30.6)
Solid	14/20 (70.0)	10/15 (66.7)	14/19 (73.7)
p	ns	ns	**0.0022**
Treatment			
On	33/59 (55.9)	24/53 (45.3)	25/56 (44.6)
Off	5/11 (45.5)	5/13 (38.5)	4/12 (33.3)
p	ns	ns	ns
Type of Treatment			
Intensive	15/27 (55.6)	13/24 (54.2)	15/28 (53.6)
Less intensive	18/32 (56.3)	11/29 (37.9)	10/28 (35.7)
p	ns	ns	ns
Doses			
One	24/49 (49.0)	21/45 (46.7)	19/45 (42.2)
Two	14/21 (66.7)	8/21 (38.1)	10/23 (43.5)
p	ns	ns	ns
Pre-vaccination Seroprotection			
<40	18/36 (50.0)	17/38 (44.7)	15/46 (32.6)
≥40	20/34 (58.8)	12/28 (42.9)	14/22 (63.6)
p	ns	ns	**0.02**
Sampling Time			
≤45*	21/31 (67.7)	16/31 (51.6)	16/33 (48.5)
>45*	17/39 (43.6)	13/35 (37.1)	13/35 (37.1)
p	0.0555	ns	ns
WBC (/mm^3)**			
Seroconversion median (range)	4000 (1400–23000)	4000 (1400–12400)	4100 (1400–23000)
non seroconversion median (range)	4300 (1200–14100)	4500 (1200–14100)	4400 (1200–14100)
p	ns	ns	ns
Lymphocytes (/mm^3)**			
≤1000	9/21 (42.9)	7/18 (38.9)	7/20 (35.0)
>1000	28/46 (60.9)	21/45 (46.7)	22/45 (48.9)
p	ns	ns	ns
ANC (/mm^3)**			
Seroconversion median (range)	2071 (546–11040)	2079 (546–8184)	2095 (546–11040)
non seroconversion median (range)	2300 (743–9207)	2204 (804–9207)	2450 (804–9207)
p	ns	ns	ns
Time after last chemo (days)			
Seroconversion: median	3	4	4
(range)	(0–180)	(0–180)	(0–180)
non Seroconversion: median	0	0	0
(range)	(0–180)	(0–180)	(0–180)
p	ns	ns	ns

ns: non significant, WBC: White Blood Cells count, ANC: Absolute Neutrophils Count, *time between final vaccination and collection of second sample, **at the time of vaccination, cases with antibody titers ≥320 before the vaccination, for any virus included in the vaccine, were excluded (5 cases for H1N1, 9 for H3N2 and 7 for B), for 3 patients, the count of lymphocytes was missing.

Previous studies have shown a range of pre-vaccination seroprotective rates from 27%-43% for H1N1, 20%-68% for H3N2 and 0–31% for B[7,8,12,23]. In our cohort, pre-vaccination seroprotective rates were 52% for H1N1, 49% for H3N2, and 39% for B strain confirming that pre-vaccination seroprotective rates were higher for influenza viruses H1N1 and H3N2 than for B. Exposure to different circulating influenza viruses and probably natural infection or immunization in the past are possible explanations for these differences.

Post vaccination seroprotective rates were found to be significantly higher for all 3 stains in comparison with pre-vaccination ones. Our results confirm an adequate immune response to influenza vaccine which is even higher for H1N1 (79%) and B strains (59%) in comparison with the corresponding published range of rates: 61–63% for H1N1, 55–85% for H3N2 (75% in our study) and 14–41% for B.[7,12] However, these rates are generally lower compared to historical healthy controls.[28,29] Moreover, the differences between pre- and post-vaccination seroprotective rates were in some studies significant for all 3 strains[12] or only for influenza B[16]. In the present study, we have also detected increased seroconversion rates: a 4-fold increase was found in 54%, 44% and 43% of vaccinated children for H1N1, H3N2 and B respectively. These findings are within the range reported in previous studies: 43–65% for

Table 5. Multivariate Analysis of HAI titers (transformed into log2) by patients' characteristics.

	Variable	Category	B	CI95	p
H1N1	HAI Pre- Vaccination		.794	.573 to 1.015	**<.001**
	ALC		.001	.000 to .001	**.029**
	Sampling Time Interval		−.015	−.029 to -.001	**.036**
H3N2	HAI Pre- Vaccination		.758	.571 to .944	**<.001**
	Malignancy	Hematological Solid	.562	.098 to 1.026	**.018**
	ANC		−.000	−.000 to −.000	**.035**
B	HAI Pre- Vaccination		.895	.699 to 1.091	**<.001**
	Malignancy	Hematological Solid	.734	.249 to 1.219	**.004**

Dependent variable: Post vaccination Hemagglutination-Inhibition antibody titers (HAI) transformed into log2, ALC: Abs`olute Lymphocytes Count, ANC: Absolute Neutrophils Count, CI95: 95% Confidence Interval for B (B: coefficient).

H1N1, 40–43% H3N2 and 33–45% for B.[7,8] We also confirmed that geometric mean titers present significant increases after vaccination for all three strains. According to Bectas et al this indicates that an increase in absolute antibody titers might confer additional protection.[11] Previous studies have shown significant increase of GMTs for all 3 strains[23] or only for H1N1 and H3N2.[7,12]

From our findings, it was also shown that the immune response was better for H1N1 and H3N2 compared to B strain. This is in accordance with previous studies suggesting an inferiority of the response to influenza B strain even among healthy individuals.[27]

In a recent meta-analysis it was shown that our results are comparable with the recent literature.[9] The differences regarding the immune response among studies included in this meta-analysis as well as in our study were noted irrespective of which method was used to evaluate the response: seroprotection – seroconversion rate or GMTs.

In our study patients, no laboratory proven influenza infection was recorded whereas in other similar studies the corresponding incidence in the vaccinated study population ranged from 0–2%.[7,8,16]

Regarding the factor analysis, variables found to be related to a better immune response included a higher ALC, older age, solid tumors, higher pre-vaccination HAI titers and shorter sampling time interval. Nevertheless, these findings were not confirmed for all three vaccine strains and for all methods of correlation. The related data in the literature are also controversial.[7,8,12,23] Especially for pre-vaccination HAI titers, several authors have noted that pre-existing titers significantly affect response to vaccines.[30] In the current study, we have shown that higher pre-vaccination titers are significantly associated with higher post-vaccination titers (tables 4,5). In contrast, Sasaki et al have shown an inverse relationship between status of previous vaccination and/or higher baseline antibody level with lower immune responses.[31] Nevertheless, Beyer et all, have found similar to our study results but only for H3N2 and they concluded that natural antibody, caused by previous infections, has a larger potential to form high post-vaccination titers, than the same amount of vaccine-induced antibody.[32]

Sampling time seems to play a role in the evaluation of immune response. No differences regarding the immune response were detected between the ≤45 & >45 subgroups irrespective of the method used (tables 2–4). On the other hand, in multiple regression analysis (Table 5), time after vaccination was confirmed as independent factor (as continuous numeric variable) for the post-vaccination GMTs, but only for the H1N1 subunit. Apparently, any correlation between time after vaccination and antibody titers reflects the durability of seroprotection. Consequently, the policy concerning the "optimal time" of influenza immunization in each setting should take into consideration the time that the annual influenza outbreak usually occurs. Nevertheless, in order to draw safe conclusions on this topic, serial measurements in the same patients should be conducted. Hakim et al have shown that the most significant decrease in titers was documented against B antigen, concluding that immunity against B antigen was more likely to be lost by 9 months after vaccination compared to H1N1 and H3N2 strains.[26] These findings also support the administration of vaccine on an annual basis.

Regarding the type of malignancy, we observed a better immune response in patients with solid tumors for some or all strains depending on the method of correlation. The literature reports are controversial and not concordant for all strains.[7,8,16,23] Differences in the nature of each type of cancer and the type of corresponding antineoplastic treatment should be taken into consideration. Regarding age, older children are more likely to have been exposed to the virus in the past and therefore develop a better immune response.[8,16,23] In our study, patients >9 years were found to have a higher post-vaccination seroprotective titer for H3N2 and B strains. Our findings, in line with the current literature, also showed an association between ANC and immune response but not for all strains and all methods of evaluation.[8,23] We also found that a higher ALC was correlated with a better response especially for the H1N1 strain and children with ALC>1000/mm^3 presented significantly higher post-vaccination seroprotection rates against all strains. However, in terms of seroconversion rates, the differences were not significant. In the past, studies have also highlighted the role of ALC in vaccine immunogenicity.[7,16]

The small size of our study and probably other confounding factors (patients in different phases of their disease, different types of cancer or chemo protocols during a certain influenza season) may explain unexpected findings such as the fact that children receiving two doses of vaccine or those being off treatment failed to achieve significantly higher post-vaccination seroprotection rates for all strains. From the review of the literature, it is clear that two vaccine doses lead to a better immune response and that prior exposure to the circulating viral strain

seems to increase the likelihood of response although these findings are not true for all strains.[6-8,18,23,26] Previous studies have also shown that off treatment patients or those receiving low intensity treatment, have a better response but not for all strains.[7,8,16] Nevertheless, our findings allow us to conclude that even during intensive treatment the response rate may be adequate at least for some patients.

Our study presents several limitations. Possible exposure to influenza virus in the past or even during the study is a potential confounder to the effect of the vaccine on the immune response. Unfortunately, this factor is not easily evaluable. In addition, no data concerning previous immunizations, before chemotherapy, were available making the interpretation of our results more difficult. It is true that analyses of immune response are complicated by patients with high pre-vaccination titers and especially when these individuals comprise a substantial portion of the sample. However, influenza immunization is not recommended for healthy children in our national immunization program and therefore only a very low percentage may have been previously vaccinated. Furthermore, it is not clear whether a HAI titer level of 40 which is considered protective in healthy children is also protective in children with cancer.[23] Regarding other limitations, no control group of healthy children was included. Nevertheless, seroprotection and seroconversion rates were not remarkably inferior to previous studies.[28,29] The different times that post-vaccination sera were collected may also influence the results, however, this practice helped us to derive indirect conclusions about the durability of the immune response. All these limitations are clearly explained by the nature of the study itself and justify why all similar studies do not always produce concordant results. The different methods used for the analyses also play a crucial role. Furthermore, the small size of our study, especially concerning subgroups, and the fact that it was conducted in a single center are additional limitations. It is obvious that larger randomized multicenter studies are required to validate our results.

On the other hand, our study has several strengths including the evaluation of the immune response to influenza vaccination by using simultaneously 3 methods: seroprotection rate, fourfold rise in antibody titer and pre- & post-vaccination GMTs in both hematological malignancies and solid tumors cases. Moreover, the role of sampling time in the evaluation of immune response has been highlighted.

In conclusion, our study supports existing recommendations concerning immunization with the trivalent inactivated influenza vaccine in children with cancer on an annual basis. A remarkable proportion of children with cancer are protected after vaccination and this is more obvious concerning H1N1 or H3N2 strains, and in children with solid tumors. Moreover, different variables were found to influence the immunogenicity whereas pre-vaccination titers play probably an important role in post-vaccination response. Protection decreases as time passes after immunization and therefore clinicians should select the optimal time of vaccination during the flu season. Consequently, the vaccine can be considered safe and effective in this high-risk population but it remains unclear if this immune response is effective in clinical terms. An additional strategy is to immunize family members and, of course, the healthcare workers.[8]

Materials and methods

The study was undertaken over the 2012–13 influenza season. We evaluated children with cancer who received influenza vaccination scheduled according to their age.

Patient eligibility

All patients (≥6 months to 17 years of age) receiving chemotherapy or within 6 months after the completion of their treatment were eligible. Recruitment was conducted during the patients' routine visits in the Oncology department of P & A Kyriakou Children's Hospital during the 2012–13 influenza period (October – February).

Patients were ineligible if they had at least one of the following characteristics: Children younger than 6 months, patients receiving induction treatment for leukemia, neutrophil count less than 500/mm^3, egg allergy or history of anaphylactic reaction to any of the vaccine ingredients, documented influenza infection prior to vaccination during the same season, intravenous immunoglobulin within the last 3 months, history of Guillain–Barré syndrome and lack of a second blood sample.

Vaccination schedule

Patients were vaccinated according to international standards with one (for patients > 9 years) or two (aged < 9 years) intramuscular doses of the vaccine, given at least 4 weeks apart, while their antineoplastic treatment was not held during the following time. Most often, patients were administered their vaccine just prior to a new chemotherapy cycle. Children were observed for any adverse event for at least one hour after each vaccination dose and parents were advised to report any sign or symptom occurring during the next days. In case of symptoms – signs of influenza disease during the next months after the vaccination, nasal swabs were collected and tested with a rapid antigen detection test.

Vaccine details

The strains included in the vaccine were A/California/7/2009 (H1N1)–like virus, A/Victoria/361/2011 (H3N2) –like virus, B/Wisconsin/1/2010-like virus. All children were vaccinated with the same vaccine commercial product (inactivated trivalent Vaxigrip vaccine, Vianex, Athens, Greece).

Serological assessment

Blood sample (at least 3 ml), was taken prior to vaccination and at least 4 weeks following the final (second) vaccination dose (in case of two doses) during the patients' routine visits. Following collection, blood samples were centrifuged and stored at −80°C until laboratory measurement of antibody titers. Serological analysis (Hemagglutination Inhibition Assay – HAI) was performed in serial dilutions of serum samples collected from these children before and after influenza vaccination in order to determine the antibody titers against each influenza subtype contained in the trivalent vaccine. Seroprotective titer

in an individual was defined as a pre- or post-vaccination HAI titer of ≥40. On the other hand, seroconversion was defined as a fourfold or greater increase in HAI antibody titer.[33]

Primary endpoints were considered the seroprotection rate determined as the proportion of patients with a titer ≥40 after the vaccination and the seroconversion rate defined as the proportion of those with an individual fourfold increase in HAI titer. Cases with antibody titers ≥320 before the vaccination, for any virus included in the vaccine, were excluded -only- from the seroconversion analysis[8](5 subjects were excluded for H1N1, 9 for H3N2 and 7 for B). Prevaccination and postvaccination geometric mean titers (GMTs) were also analyzed. For the needs of GMTs analysis, in cases with antibody titers less than 10, a value of 5 was accepted and used.[11]

Patients characteristics

Demographic characteristics, underlying disease, type and time of treatment, laboratory evaluation (Complete Blood Count with differential), local or systemic adverse effects as well as the compliance rate with the recommendation for vaccination were all recorded. Any influence of demographic, clinical and laboratory characteristics, including the number of doses on seroprotection and / or seroconversion rates, were secondary endpoints in our analysis. Patients participating in the study were administered different types of chemotherapy at varying time points and, in addition, many of them lived in other parts of the country. For these reasons, post-vaccination samples were collected at different time intervals when patients returned for chemotherapy (convenience samples) but not earlier than 4 weeks after the final vaccine dose. For that reason, additional analyses took place in order to evaluate the influence of this factor as categorical (≤45 days vs >45 days) or continuous variable in our results.

Ethical issues

Ethical approval for the study was obtained from the hospital ethics committee and written informed consent was obtained from the parents of each child prior to recruitment.

Statistics

Analysis was done by using "Statistical Package for Social Sciences" (SPSS, version 18). Comparison of categorical and continuous variables was performed with Fisher exact test, chi-square, McNemar and Mann–Whitney test respectively. Antibody titers were transformed into log2 for the analysis of GMTs and comparisons between pre- and post-vaccination values were performed by using the Wilcoxon signed rank test. Multivariate linear regression analysis was used for multivariate analyses. Statistical significance was considered as $p<0.05$.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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