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An open-label, randomized clinical trial assessing immunogenicity, safety and tolerability of pandemic influenza A/H1N1 MF59-adjuvanted vaccine administered sequentially or simultaneously with seasonal virosomal-adjuvanted influenza vaccine to paediatric kidney transplant recipients

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Abstract

Background. The aim of this study was to investigate the immunogenicity, safety and tolerability of the 2009 A/H1N1 MF59-adjuvanted influenza vaccine, administered sequentially or simultaneously with the seasonal 2009–10 virosomal-adjuvanted influenza vaccine, to paediatric kidney transplant recipients.

Methods. Thirty-two children and adolescents with transplanted kidneys and 32 age- and gender-matched healthy controls were randomized 1:1 to receive the pandemic vaccine upon enrolment and the seasonal vaccine 1 month later (16 transplant recipients and 16 healthy controls), or to receive the two vaccines simultaneously upon enrolment (16 transplant recipients and 16 healthy controls).

Results. When the pandemic vaccine was administered sequentially to the seasonal vaccine, it was significantly less immunogenic in the patients than in the controls ($P < 0.05$); when it was administered together with the seasonal vaccine, the immune response of both patients ($P < 0.05$) and controls ($P < 0.05$) was significantly greater than when it was administered sequentially. Seroconversion rates and the geometric mean titres of all of the seasonal antigens were significantly lower in the patients, regardless of the type of vaccine administration ($P < 0.05$). Simultaneous administration was associated with a better immune response against A/H1N1 and A/H3N2 antigens in both patients and controls, and did not increase the mild local and systemic reactions. No impact on renal function was observed.

Conclusions. Paediatric kidney transplant recipients have a lower immune response to the pandemic influenza A/H1N1 MF59-adjuvanted and seasonal virosomal-adjuvanted influenza vaccines than healthy controls. The simultaneous administration of the two vaccines seems to increase immune response to both pandemic and seasonal A/H1N1 and A/H3N2 antigens, and has the same safety profile as that of the pandemic vaccine administered sequentially to the seasonal vaccine.

Keywords: A/H1N1 influenza vaccine; kidney transplantation; pandemic influenza; paediatrics; seasonal influenza vaccine

Introduction

Not only influenza has been associated with acute kidney allograft rejection [1], but also kidney transplant recipients are at higher risk of experiencing severe infection because of the potent immunosuppressive therapy they receive to prevent the rejection itself [2,3]. Consequently, also considering that recent literature does not support the concerns that vaccination may trigger rejection [4], it is strongly recommended that kidney transplant recipients be included in the list of subjects who should be administered the seasonal trivalent-inactivated vaccine against influenza viruses every year [5,6]. However, there are conflicting reports concerning the immunogenicity and efficacy of the vaccine in such patients [7–13].

In April 2009, the emergence of influenza virus A/H1N1 in humans caused the first influenza pandemic since 1968 [14] and led to the preparation of various specific vaccines with or without adjuvant [15]. Immunosuppressed patients (including kidney transplant recipients) were included among the subjects having a right to priority immunization [16,17], but this decision was not supported by any specific studies of the vaccines' immunogenicity,

safety and tolerability in these patients. Moreover, although it was suggested that solid-organ transplant recipients should receive both the pandemic and the seasonal influenza vaccine [18], no information is available as to whether the simultaneous administration of pandemic and seasonal influenza virus antigens can influence immune response and/or have a negative impact on transplanted kidneys. This is all more important because the influenza vaccine specifically prepared for the 2010–11 influenza season contains both the new 2009 A/H1N1 pandemic virus and two old seasonal A/H3N2 and B viruses.

The aim of this study was to verify the immunogenicity, safety and tolerability of the 2009 A/H1N1 MF59-adjuvanted influenza vaccine, administered sequentially or together with the seasonal 2009–10 virosomal-adjuvanted influenza vaccine to paediatric kidney transplant recipients.

Materials and methods

Study population

This open-label study, which started on 1 November 2009, involved 32 paediatric kidney transplant recipients who were being regularly followed up at the outpatient clinic of the Pediatric Nephrology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, and who had never previously been vaccinated against seasonal influenza. As the pandemic A/H1N1 influenza vaccine only became available in Italy ~4 weeks after the first documented episode of pandemic influenza, the transplant recipients who had experienced an influenza-like illness in 4 weeks preceding the start of the study were excluded in order to avoid the risk of enrolling already infected subjects. The control group consisted of the same number of healthy age- and gender-matched subjects selected using the same criteria and enrolled sequentially among those who attended the outpatient clinic for a control visit after a previous hospitalization for minor surgical problems.

The members of each group were randomly assigned 1:1 on the basis of a computer-generated randomization list to receive the pandemic vaccine upon enrolment and the seasonal vaccine 4 weeks (28 ± 2 days) later, or to receive the two vaccines simultaneously upon enrolment.

The study was approved by the Ethics Committee of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, and written informed consent was obtained from the patients' parents or legal guardians, and from the patients themselves.

Vaccines

The pandemic vaccine was the monovalent pandemic influenza A/H1N1 MF59-adjuvanted vaccine Focetria (Novartis, Siena, Italy); each 0.5-mL dose contained 7.5 μg of H1 haemagglutinin, 9.75 mg of the squalene MF59, 1.175 mg of polysorbate 80 and 1.175 mg of sorbitan trioleate in buffer. The seasonal vaccine was the 2009–10 virosomal-adjuvanted seasonal influenza vaccine produced by Crucell (Leiden, the Netherlands); each 0.5-mL dose contained 15 μg each of A/Brisbane/59/2007 (H1N1)-like, A/Brisbane/10/2007 (H3N2)-like and B/Brisbane/60/2008-like purified influenza surface antigens that were neuraminidase- and haemagglutinin-integrated into the lipid membrane of the virosome.

Procedures

Between 1 November 2009 and 15 November 2009, all of the enrolled subjects underwent a baseline assessment that included recording of demographic and medical history data, and a physical examination. Demographic data of each transplant recipient included the type of graft donor, the number of transplants, the aetiology of end-stage primary renal disease, the time interval between transplantation and vaccination, and the immunosuppressive regimen. Blood samples were drawn from all of the children at enrolment, and 4 weeks (28 ± 2 days) and 8 weeks (56 ± 2 days) after each vaccine administration, to evaluate immunogenicity and blood urea nitrogen (BUN) and creatinine concentrations; at the same visits, a urine sample was collected for complete laboratory analysis.

The pandemic vaccine was injected into the left deltoid muscle and the seasonal vaccine in the right deltoid muscle, regardless of the time of administration.

The subjects were observed for 30 min after the injection, and they or their parents recorded the occurrence of solicited and unsolicited local symptoms (erythema, swelling/induration and pain) or systemic symptoms (an axillary temperature of ≥38°C, irritability, sleepiness, changes in eating habits, vomiting, diarrhoea, malaise and muscle aches) for the next 14 days. The symptoms were considered mild if they did not interfere with normal everyday activities, and severe if they prevented them and required medical attention. Adverse reactions were defined as any reaction that persisted for longer than 7 days after the vaccination, and serious adverse reactions as any reaction that required medical attention or hospitalization during the study period.

The subjects and their parents were asked to pay particular attention to the development of symptoms resembling influenza-like illness throughout the study period and, if these appeared, to return immediately to the study centre for clinical evaluation and laboratory testing of nasal swabs for influenza viruses [19]. Further data regarding the clinical events that occurred between vaccine administrations were collected during the visits made for vaccine administration and/or blood collection.

Immunogenicity was evaluated by measuring haemagglutination-inhibiting (HI) antibodies to the influenza strains contained in the vaccines using standard assays [20]. The serum samples were tested in duplicate at an initial dilution of 1:10, and those that were negative for the antibody were assigned an arbitrary titre of 1:5. HI antibody titres were expressed as the reciprocal of the highest serum dilution that completely inhibited haemagglutination.

Humoral immune response was assessed on the basis of the seroconversion rate (defined as the percentage of subjects experiencing at least a 4-fold increase in a seropositive pre-vaccination HI antibody titre, or an increase from <10 to ≥40 in those who were seronegative), geometric mean titres (GMTs), the difference between the mean pre- and post-vaccination titres, and the seroprotection rate (defined as the percentage of subjects reaching an HI titre of ≥40).

Statistical analysis

Comparisons were made between the kidney transplant recipients and the healthy controls, and between the groups vaccinated sequentially or simultaneously. With a 5% type 1 error rate and a power of 90%, 16 transplant recipients and 16 healthy controls were required to show a difference of 50% in immunogenicity.

Continuous variables are expressed as mean values ± standard deviation (SD), and categorical variables as numbers and percentages. The continuous data were analysed using Student's *t*-test if they were normally distributed (on the basis of the Shapiro–Wilk statistic) or a Wilcoxon rank-sum test if they were not. The categorical data were analysed using contingency tables and the chi-square or Fisher's test, as appropriate. All of the analyses were two-tailed, and P-values of <0.05 were considered significant.

Results

Study population

Table 1 shows the demographic and clinical characteristics of the study population. Thirty-two kidney transplant recipients (20 males; mean age ± SD 15.5 ± 5.5 years) all received kidneys from deceased donor and underwent only one transplant. The most frequent cause of the end-stage renal disease (>50%) leading to transplantation was congenital kidney and urinary tract anomalies. The mean time between transplantation and vaccination was 94.7 ± 63.4 months. More than 80% of the cases were receiving calcineurin inhibitor-based immunosuppressive treatment

Table 1. Demographic and clinical characteristics of the study population

Characteristic	Pandemic vaccine alone followed by seasonal vaccine alone		Pandemic vaccine + seasonal vaccine	
	Transplant recipients (n = 16)	Healthy controls (n = 16)	Transplant recipients (n = 16)	Healthy controls (n = 16)
Age (years), mean ± SD	15.4 ± 5.6	15.6 ± 5.5	15.7 ± 5.5	15.4 ± 5.7
Males, n (%)	10 (62.5)	10 (62.5)	10 (62.5)	10 (62.5)
Caucasians, n (%)	13 (81.3)	13 (81.3)	12 (75.0)	12 (75.0)
Antibiotic treatment in previous 3 months, n (%)	1 (6.3)	1 (6.3)	1 (6.3)	1 (6.3)
Primary renal disease, n (%)				
Congenital anomalies of the kidney and urinary tract	9 (56.3)	NA	9 (56.3)	NA
Congenital nephritic syndrome	1 (6.3)	NA	3 (18.7)	NA
Nephronophthisis	2 (12.5)	NA	0 (0.0)	NA
Neurological bladder	1 (6.3)	NA	1 (6.3)	NA
Interstitial nephritis	1 (6.3)	NA	1 (6.3)	NA
Kidney thrombosis	0 (0.0)	NA	1 (6.3)	NA
Wilms' tumour	0 (0.0)	NA	1 (6.3)	NA
Haemolytic uraemic syndrome	1 (6.3)	NA	0 (0.0)	NA
Cortical necrosis	1 (6.3)	NA	0 (0.0)	NA
Time between transplant and vaccines, months (mean ± SD)	96.9 ± 78.7	NA	93.6 ± 53.4	NA
Immunosuppressive regimens, n (%)				
Tacrolimus-based	10 (62.5)	NA	9 (56.3)	NA
Cyclosporine-based	4 (25.0)	NA	4 (25.0)	NA
Sirolimus-based	2 (12.5)	NA	3 (18.7)	NA
Hospitalized in previous 3 months, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Previously administered seasonal influenza vaccine, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

No significant between-group difference.NA, not applicable.

(tacrolimus or cyclosporine). All the patients had received induction therapy with basiliximab after transplantation.

Sixteen of the subjects in both groups received the pandemic vaccine upon enrolment and the seasonal vaccine 4 weeks later; the other 16 in both groups received both vaccines at the same time.

None of the subjects in either group had a history of influenza-like illness since May 2009 (date of circulation of pandemic influenza A/H1N1 virus in Europe) or experienced an episode of laboratory-confirmed pandemic or seasonal influenza during the study period.

Immune response

Table 2 shows the data regarding the immunogenicity of the monovalent pandemic influenza A/H1N1 MF59-adjuvanted vaccine administered simultaneously with, or 1 month before the seasonal vaccine. Some of the subjects

Table 2. Immunogenicity end points against the 2009 pandemic A/H1N1 influenza strain in kidney transplant recipients and healthy controls

Values and timelines	Pandemic vaccine alone followed by seasonal vaccine alone		Pandemic vaccine + seasonal vaccine	
	Transplant recipients (n = 16)	Healthy controls (n = 16)	Transplant recipients (n = 16)	Healthy controls (n = 16)
Seroconversion, n (%)				
Baseline	NA	NA	NA	NA
28 ± 2 days post-Dose 1	4 (25.0)°	16 (100.0)	15 (93.8)^	15 (100.0)
56 ± 2 days post-Dose 1	7 (43.7)°	16 (100.0)	16 (100.0)^	15 (100.0)
GMT (fold increase)				
Baseline	31.56 (NA)	44.16 (NA)*	38.75 (NA)*	57.63 (NA)*
28 ± 2 days post-Dose 1	48.13 (1.5)°	270.33 (6.1)	426.30 (11.0)^	630.71 (10.9)°
56 ± 2 days post-Dose 1	46.25 (1.5)°	291.82 (6.6)	530.0 (13.7)^	643.10 (11.2)°
Seroprotection, n (%)				
Baseline	5 (31.2)	7 (43.7)*	6 (37.5)*	7 (43.7)*
28 ± 2 days post-Dose 1	10 (62.5)	16 (100.0)	15 (93.8)	16 (100.0)
56 ± 2 days post-Dose 1	13 (81.3)	16 (100.0)	16 (100.0)	16 (100.0)

GMT, geometric mean titres; NA, not applicable. °$P < 0.05$ vs healthy controls administered pandemic vaccine alone followed by seasonal vaccine alone. *$P < 0.05$ vs 28 ± 2 days post-Dose 1 and vs 56 ± 2 days post-Dose 1. ^$P < 0.05$ vs transplant recipients administered pandemic vaccine alone followed by seasonal vaccine alone.

in both groups had measurable specific antibody titres against the pandemic virus at the time of enrolment. Subjects with measurable specific antibody titres were enrolled in the same period as those without. When administered alone, the pandemic vaccine was significantly less immunogenic in the transplant recipients than in the healthy controls ($P < 0.05$). When it was administered together with the seasonal vaccine, the immune response of both patients ($P < 0.05$) and controls ($P < 0.05$) was significantly greater than when it was administered alone; this effect was significantly more pronounced in the case of the transplant recipients, whose immune response became quite similar to that of the healthy controls.

Table 3 shows the data regarding the immunogenicity of the seasonal virosomal-adjuvanted vaccine administered simultaneously with, or 1 month after the pandemic vaccine. Once again, some of the enrolled subjects already had detectable specific antibody titres against the influenza antigens included in the seasonal vaccine. The seroconversion rates and GMTs of all of the antigens were significantly lower in the transplant recipients than in the healthy controls regardless of the time of administration ($P < 0.05$). In both groups, simultaneous administration was associated with a better immune response against A/H1N1 and A/H3N2 than that observed when the vaccines were administered separately, but the difference was statistically significant only in the control group ($P < 0.05$).

General safety and tolerability

Table 4 summarizes the solicited and unsolicited local and systemic symptoms observed in 14 days following the administration of each vaccine. Between 18.8% and 25.0% of the children experienced at least one local reaction, with no difference between the groups. All of these local reactions were classified as mild, and their frequency was not increased by the simultaneous administration of the two vaccines. Systemic reactions occurred in ~12% of the children, with no significant difference between the groups. Once again, they were all mild, and their frequency was not increased by simultaneous vaccine administration. No severe adverse event was recorded in either group.

Impact on renal function

Table 5 shows the data regarding BUN and serum creatinine levels, and the results of the urine analyses, in the kidney transplant recipients before, and 1 and 3 months after the administration of the vaccines. There were no differences in any of the studied variables regardless of the schedule of administration.

Discussion

The results of this study show that, especially when administered separately, the pandemic and seasonal influenza vaccines are less immunogenic in paediatric kidney transplant recipients receiving (mainly calcineurin inhibitor-based) immunosuppressive therapy than in healthy subjects even when they are given several after transplantation.

Table 3. Immunogenicity end points against seasonal strains in kidney transplant recipients and healthy controls

	Pandemic vaccine alone followed by seasonal vaccine alone						Pandemic vaccine + seasonal vaccine					
	Transplant recipients ($n = 16$)			Healthy controls ($n = 16$)			Transplant recipients ($n = 16$)			Healthy controls ($n = 16$)		
Values and timelines	A/H1N1	A/H3N2	B	A/H1N1	A/H3N2	B	A/H1N1	A/H3N2	B	A/H1N1	A/H3N2	B
Seroconversion, *n* (%)												
Baseline	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
28 ± 2 days post-dose 1	2 (12.5)^	2 (12.5)^	0 (0.0)^	14 (87.5)	14 (87.5)	14 (87.5)	4 (25.0)*	4 (25.0)*	3 (18.8)*°	15 (93.8)	15 (93.8)	14 (87.5)
56 ± 2 days post-dose 1	3 (18.8)^	3 (18.8)^	1 (6.3)^	15 (93.8)	15 (93.8)	15 (93.8)	5 (31.3)*	6 (37.5)*	3 (18.8)*°	16 (100.0)	16 (100.0)	14 (87.5)
GMT (fold increase)												
Baseline	27.81 (NA)	28.13 (NA)	22.81 (NA)	25.82 (NA)#	57.61 (NA)#	15.61 (NA)#	32.5 (NA)#	47.81 (NA)	13.13 (NA)	39.31 (NA)#	31.19 (NA)#	15.83(NA)#
28 ± 2 days post-dose 1	43.13 (1.6)^	51.25(1.8)^	36.25 (1.6)^	233.69 (9.1)	255.16 (4.4)	58.19 (3.7)	109.38 (3.4)*	70.63 (1.5)*	26.88 (2.0)*	733.76 (18.7)^	491.52 (15.8)^	61.16 (3.9)
56 ± 2 days post-dose 1	51.25 (1.8)^	59.38 (2.1)^	28.75 (1.3)^	249.36 (9.7)	260.36 (4.5)	55.39 (3.5)	117.61 (3.6)*	84.38 (1.8)*	23.13 (1.8)*	810.16 (20.6)^	449.39 (14.4)^	63.76 (4.0)
Seroprotection, *n* (%)												
Baseline	5 (31.3)	5 (31.3)	2 (12.5)	5 (31.3)#	5 (31.3)#	3 (18.8)#	6 (37.5)	6 (37.5)	1 (6.3)	5 (31.3)#	6 (37.5)#	1 (6.3)#
28 ± 2 days post-dose 1	7 (43.8)	8 (50.0)	4 (25.0)	14 (87.5)	15 (93.8)	15 (93.8)	11 (68.8)	10 (62.5)	5 (31.3)	15 (93.8)	14 (87.5)	14 (87.5)
56 ± 2 days post-dose 1	7 (43.8)	8 (50.0)	5 (31.3)	15 (93.8)	15 (93.8)	15 (93.8)	12 (75.0)	12 (75.0)	5 (31.3)	16 (100.0)	15 (93.8)	15 (93.8)

NA, not applicable. ^$P < 0.05$ vs healthy controls administered pandemic vaccine alone followed by seasonal vaccine alone. #$P < 0.05$ vs 28 ± 2 days post-dose 1 and vs 56 ± 2 days post-dose 1. *$P < 0.05$ vs healthy controls administered pandemic vaccine + seasonal vaccine. °$P < 0.05$ vs transplant recipients administered pandemic vaccine alone followed by seasonal vaccine alone.

Table 4. Summary of solicited local and systemic reactions in 14 days following vaccination with the 2009 pandemic A/H1N1 MF59-adjuvanted influenza vaccine administered together with or 1 month before the seasonal virosomal-adjuvanted influenza vaccination to kidney transplant recipients and healthy controls

	Sequential vaccine administrations				Simultaneous vaccine administrations	
	Pandemic alone		Seasonal alone		Pandemic + seasonal	
Adverse events	Transplant recipients (n = 16)	Healthy controls (n = 16)	Transplant recipients (n = 16)	Healthy controls (n = 16)	Transplant recipients (n = 16)	Healthy controls (n = 16)
Local reactions, n (%)						
Erythema	0 (0.0)	1 (6.3)	1 (6.3)	1 (6.3)	1 (6.3)	1 (6.3)
Swelling/induration	3 (18.8)	2 (12.5)	2 (12.5)	1 (6.3)	4 (25.0)	2 (12.5)
Pain	3 (18.8)	2 (12.5)	2 (12.5)	2 (12.5)	4 (25.0)	2 (12.5)
At least one local event	4 (25.0)	4 (25.0)	3 (18.8)	3 (18.8)	4 (25.0)	3 (18.8)
Systemic reactions, n (%)						
Fever ≥38°C	1 (6.3)	1 (6.3)	0 (0.0)	1 (6.3)	1 (6.3)	1 (6.3)
Rhinitis	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Malaise	1 (6.3)	0 (0.0)	1 (6.3)	1 (6.3)	1 (6.3)	0 (0.0)
Sleepiness	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Changed eating habits	0 (0.0)	1 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.3)
Vomiting	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Diarrhoea	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
At least one systemic event	2 (12.5)	2 (12.5)	1 (6.3)	2 (12.5)	2 (12.5)	2 (12.5)
At least one local or systemic event	4 (25.0)	4 (25.0)	3 (18.8)	4 (25.0)	5 (31.3)	4 (25.0)
Required drugs for local or systemic events	2 (12.5)	1 (6.3)	1 (6.3)	1 (6.3)	3 (18.8)	2 (12.5)
Serious adverse events	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Percentages in parentheses. No significant differences between the transplant recipients and healthy controls.

However, the immune response to the pandemic antigen is greater than that to the seasonal antigens, as demonstrated by the larger number of subjects achieving seroprotection and the higher antigen GMTs 4 and 8 weeks after immunization, despite the fact that the antigen concentration in the pandemic MF59-adjuvanted vaccine was half the usual concentration of each of the antigens in traditional seasonal vaccines. It is unlikely that this effect was attributable to previous or concomitant infection with the wild pandemic virus because the GMTs also increased in the subjects without serum-detectable immunity at the time of enrolment, and because careful clinical monitoring of both patients and controls reduced the possibility of a response due to an infection occurring during the study period. In our opinion, it may have been related to the more immunogenic nature of the 2009 influenza A/H1N1 haemagglutinin [21–24] and the fact that the pandemic vaccine contained MF59, an adjuvant that can significantly increase immune responses to influenza antigens even in young patients in whom they are known to be weaker [25,26].

The very low immune response evoked by the seasonal vaccine is in line with the findings of other authors showing that transplant patients have impaired immune responses to a number of vaccines (including seasonal influenza vaccine), particularly when they are receiving calcineurin

Table 5. Impact on renal function in the months following vaccination with the 2009 pandemic A/H1N1 MF59-adjuvanted influenza vaccine administered together with or 1 month before the seasonal virosomal-adjuvanted influenza vaccination to kidney transplant recipients

	Baseline		1 month after vaccination		3 months after vaccination	
Laboratory data	Sequential group Transplant recipients (n = 16)	Simultaneous group Transplant recipients (n = 16)	Sequential group Transplant recipients (n = 16)	Simultaneous group Transplant recipients (n = 16)	Sequential group Transplant recipients (n = 16)	Simultaneous group Transplant recipients (n = 16)
BUN, mg/dL						
Mean values ± SD	61.0 ± 28.9	63.5 ± 22.6	61.5 ± 29.3	63.1 ± 21.7	61.3 ± 29.1	63.3 ± 22.3
Serum creatinine, mg/dL						
Mean values ± SD	1.29 ± 0.53	1.43 ± 0.69	1.27 ± 0.53	1.38 ± 0.77	1.30 ± 0.49	1.40 ± 0.58
Proteinuria, mg/day	125 ± 25	119 ± 30	128 ± 24	120 ± 28	133 ± 24	113 ± 39
Proteinuria/creatininuria	0.10	0.11	0.11	0.13	0.9	0.14
Haematuria, n. (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Percentages in parentheses. No significant differences between baseline, 1 month and 3 months after vaccination.BUN, blood urea nitrogen.

inhibitor-based immunosuppressive therapy like our patients [11–13,27]. However, it is unlikely that induction therapy received after transplantation has influenced the vaccine response, because all the patients received it several months before the study and, in other conditions like cancer, it has been demonstrated that the strong effect of induction therapy is finished a few months after its end [28]. Moreover, all of our patients received basiliximab as induction therapy, and it is known that the long-term effects of this drug are lower than that of thymoglobulin [29,30].

Interestingly, the immune response of both patients and controls to the MF59-adjuvanted pandemic vaccine was significantly increased by its simultaneous administration with the seasonal virosomal-adjuvanted vaccine. In this case, the response of the transplant recipients was quite similar to that of the healthy subjects, leading to optimal seroconversion and seroprotection rates, and GMTs that were high enough to suggest long-term protection. The incremental effect of simultaneous administration also extended to the seasonal A/H1N1 and A/H3N2 antigens, although the response of the transplant recipients remained significantly weaker than that of the healthy subjects and cannot be considered satisfactory. In this regard, our findings are different from those of Vaio *et al*. that did not observe any increase in immune responses when the pandemic and seasonal influenza vaccines were administered together [31]. Their data are not directly comparable with ours because they used a pandemic vaccine containing a different adjuvant and lower antigen levels and a non-adjuvanted seasonal vaccine. Nevertheless, one possible reason for the difference is our simultaneous administration of two adjuvants. In that regard, the interaction between MF59 and virosomal adjuvants has been nicely described in a mouse model by Radosevic *et al*. [32]. Another possible explanation is the fact that, although different, the pandemic and seasonal influenza A viruses have some genetic similarities, and so, their simultaneous administration may lead to greater antigen stimulation [33]. However, it is not clear why the additive effect should be more pronounced for the pandemic than the seasonal vaccine.

Genetic similarities between the pandemic A/H1N1 and the seasonal A/H1N1 viruses may explain why a part of the study population had measurable specific antibody titres against the pandemic virus at baseline. On the other hand, although subclinical infections are possible, our study patients had no history of influenza-like illness possibly related to the pandemic virus, but considering their mean age, all of them had probably had previous history of seasonal influenza infection. This finding is in line with the data showing that population was not fully naïve to the current pandemic virus [34] and that cross-reactivity of HI antibodies is possible between pandemic A/H1N1 and seasonal A/H1N1 viruses [33].

The fact that none of our study patients had been previously vaccinated against seasonal influenza, despite the fact that transplant recipients are included among the high-risk groups for whom influenza vaccination is recommended, is not surprising and is in line with our previous data showing a very low influenza vaccination coverage among Italian high-risk categories [35].

The seasonal vaccine specifically prepared for the 2010–11 influenza season contains the pandemic antigen and the seasonal A/H3N2 and B virus antigens, but as it will not be adjuvanted (or will contain only a single adjuvant), it is difficult to foresee whether it will lead to an additive effect against the pandemic A/H1N1 antigen or whether it will adequately protect kidney transplant recipients. Consequently, given their weaker immune response in comparison with healthy controls, they need to be vaccinated and also closely monitored for influenza-like illness during the influenza period [36].

We found that the safety and tolerability of both the MF59-adjuvanted pandemic vaccine and the seasonal virosomal-adjuvanted vaccine were good in all of our study subjects, and their simultaneous administration did not increase the incidence of solicited and unsolicited local or systemic reactions in either group. Furthermore, our monitoring of the markers of renal function and the urinalyses did not reveal any signs that the pandemic vaccine might be associated with kidney graft rejection even when it was administered together with the seasonal vaccine.

In conclusion, our data indicate that the immune response of paediatric kidney transplant recipients to the MF59-adjuvanted pandemic influenza A/H1N1 vaccine and the seasonal virosomal-adjuvanted influenza vaccine is less than that of healthy controls. The simultaneous administration of the two vaccines leads to a statistically significant increase in immune response to the pandemic antigen, and a slightly higher response to seasonal A/H1N1 and A/H3N2 antigens, with no difference in safety profile. Further studies are needed to verify whether the inclusion of the pandemic antigen in the seasonal influenza vaccine specifically prepared for the 2010–11 influenza season improves the immune response of kidney transplant recipients to all influenza virus antigens.

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Conflict of interest statement. None declared.

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