Influenza A/H1N1 Vaccine in Patients Treated by Kidney Transplant or Dialysis: A Cohort Study

Nilufer E. Broeders, Anneleen Hombrouck,† Anne Lemy, Karl Martin Wissing,‡ Judith Racapé,* Karine Gastaldello,* Annick Massart,* Steven Van Gucht,† Laura Weichselbaum,* Aurelie De Mul,* Bernard Brochier,† Isabelle Thomas,† and Daniel Abramowicz**

Summary

Background and objectives In 2009, the pandemic influenza A/H1N1 accounted for worldwide recommendations about vaccination. There are few data concerning the immunogenicity or the security of the adjuvanted-A/H1N1 vaccine in transplanted and hemodialyzed patients.

Design, setting, participants, & measurements Sera from 21 controls, 53 hemodialyzed (HD) patients, and 111 renal transplant recipients (RT) were sampled before (T0) and 1 month after (T1) a single dose of Pandemrix® vaccine (GSK Biologicals, AS03-adjuvanted). We measured the neutralizing antibodies against A/H1N1/2009, the geometric mean (GM) titers, the GM titer ratios (T1/T0) with 95% confidence intervals, and the seroconversion rate (responders: ≥4-fold increase in titer). The HLA and MICA immunization was determined by Luminex technology.

Results The GM titer ratio was 38 (19 to 78), 9 (5 to 16), and 5 (3 to 6) for controls, HD patients, and RT patients, respectively ($P < 0.001$). The proportion of responders was 90%, 57%, and 44%, respectively ($P < 0.001$). In RT patients, the prevalence of histocompatibility leukocyte antigen (HLA) class I, histocompatibility leukocyte antigen class II, and MHC class I-related chain A immunization, was, respectively, 15%, 14%, and 14% before and 14%, 14%, and 11% after vaccination ($P = 1$, 1, and 0.39).

Conclusions The influenza A/H1N1-adjuvanted vaccine is of limited efficacy but is safe in renal disease populations. The humoral response is lower in transplanted *versus* hemodialyzed patients. Further studies are needed to improve the efficacy of vaccination in those populations.

Clin J Am Soc Nephrol 6: 2573–2578, 2011. doi: 10.2215/CJN.04670511

*Erasme Hospital, Department of Nephrology, Université Libre de Bruxelles, Brussels, Belgium; †National Influenza Centre, Communicable and Infectious Diseases, Scientific Institute of Public Health, Brussels, Belgium; and ‡Universitair Ziekenhuis, Department of Nephrology, Brussels, Belgium

Correspondence: Dr. Nilufer Broeders, Renal Transplantation Clinic, Hospital Erasme, Route de Lennik, 808, 1070 Brussels, Belgium. Phone: 3225553334; Fax: 3225556499; E-mail: broedersnilufer@gmail.com

Introduction

Annual seasonal influenza vaccination is strongly recommended in patients with kidney transplants and chronic kidney disease (1,2). In April 2009, a novel influenza A virus, A/California/7/2009 (H1N1) caused a new pandemic (3). Severe illness occurred in "low-risk" subjects without previous immunity, because no cross-reactive antibody was found in their sera against the new A/H1N1 strain (4). In this setting, the World Health Organization recommended a worldwide vaccination, targeting high-risk patients including those with kidney transplants and chronic kidney disease (1,5). Either a single dose of 3.75 μg of adjuvanted or 15 μg of nonadjuvanted vaccine resulted in sero-conversion in approximately 75% to 90% of healthy volunteers (6,7). In solid organ recipients (8–11) and dialysis patients (12), the overall rate of seroconversion to the seasonal flu vaccine is in the range of 30% to 50%. The use of adjuvanted vaccine has raised concerns for immunocompromised patients, because of possible induction of allo-immune responses, such as anti-human histocompatibility leukocyte antigen (HLA) antibodies (Abs) or MHC class I-related chain A (MICA) Abs (13–16). Indeed, the AS03 adjuvant present in some vaccines promotes immunogenicity by modulating the expression of local cytokines and by increasing the antigen loading in monocytes (17). Although this adjuvant has been safely administered with the H1N1 and the H5N1 strains to thousands of healthy adults (18), more data are needed about the potential for allosensitization among patients with renal disease. Indeed, many reports show that *de novo* occurrence of post-transplant anti-HLA Abs is associated with chronic rejection and graft loss (19–21). With regard to MICA antibodies, their formal pathogenic role on graft outcomes is less well established, but we thought it was worthwhile to assay them (14–16). Here, we report on the immunogenicity and safety of a single dose of adjuvanted vaccine in hemodialyzed patients and renal transplant recipients. Safety was investigated by the proportion of patients who display anti-HLA and MICA Abs prior and after vaccination.

Materials and Methods

Patients

The study was approved by our institution's ethics committee, and the authors were notified to adhere to the Declaration of Helsinki. All participants signed a

written informed consent before inclusion. Vaccination was proposed to all patients who attended our outpatient renal transplant clinic during November 2009 ($n = 235$) and who were on chronic hemodialysis in our center at that time ($n = 95$). Exclusion criteria were transplantation within 1 month, ongoing infection, leukopenia ($<2500/\mu l$), intravenous immunoglobulins injection within 3 months, and refusal of the patient. We enrolled 111 renal transplant (RT) recipients, 53 chronic kidney disease patients undergoing hemodialysis (HD), and 21 controls who were all healthy members of the hospital staff. All subjects were vaccinated between October and December 2009 with the commercially available H1N1 influenza vaccine (Pandemrix®; GlaxoSmithKline Biologicals, Rixensart, Belgium), for the first time. It contains 3.75 μg of split inactivated strain A/California/07/2009 (H1N1), together with the AS03 adjuvant that contains squalen, DL-α-tocopherol and polysorbate 80. Blood samples were collected before (T0) and 1 month after (T1) a single vaccination that was performed in the deltoid muscle. Serum samples were stored at −20°C. The scientists who performed the serologic analyses were unaware of the origin of the samples. Seasonal influenza vaccination was performed at least 1 month after H1N1 vaccination. Of note, the vast majority of our patients had also been vaccinated against seasonal influenza in 2008 with the subunit virion influenza vaccine (Influvac S®; Solvay Pharma, Brussels, Belgium). This trivalent vaccine contained purified hemagglutinin and neuraminidase of the influenza strains A/Brisbane/59/2007 (H1N1), A/Brisbane/10/2007 (H3N2), and B/Florida/4/2006. The following patient characteristics were recorded: age, gender, immunosuppressive therapy, duration of prior dialysis or transplantation, diabetes (defined as patients treated by oral therapy or insulin), and renal function according to Modification of Diet in Renal Disease categories.

Anti-H1N1 Antibody Detection

Serum samples were obtained on days 0 (T0) and 30 (T1) for antibody titration against the influenza A/California/7/2009 (H1N1) using a neutralization assay. As described previously, it is based on the ability of antibodies to inhibit the infection of a Madin-Darby canine kidney (MDCK) cell culture by the influenza virus (22). Briefly, 1:2 serial dilutions of inactivated serum samples were preincubated with a standardized amount of virus ($100TCID_{50}$) before the addition of MDCK cells (25,000 cells/well). After overnight incubation, an ELISA was used to measure influenza A viral nucleoprotein's levels in infected MDCK cells. Because serum antibodies to the influenza virus inhibit the viral infection of MDCK cells, the optical density (OD) results of the ELISA are inversely proportional to the serum antibody concentration. The initial dilution and lower detection limit of this assay was 1:10. Appropriate negative and positive control serum samples were included in all analyses. Samples were tested in duplicate in each assay, and assays were independently repeated once. The titer analyzed was the geometric mean (GM) of these four test results, expressed as the reciprocal of the strongest serum dilution with OD_{490} value less than X, where $X = [(\text{average } OD_{490} \text{ of virus control wells}) - (\text{average } A_{490} \text{ of cell control wells})]/2 + (\text{average } OD_{490} \text{ of cell control wells})$ (16). Samples without detectable antibody activity were assigned the titer of half the assay detection limit (1:5). Although this assay reflects the ability of the patient's serum to prevent viral infection of cells, there is no formal titer cutoff for seroprotection. To calculate GM titers, the reciprocal of serum dilutions was log transformed to base 10 as recommended (23,24). This enabled us to calculate summary statistics such as means and confidence intervals (CIs), which were then retransformed into titer equivalents (geometric mean titers) by exponentiation. GM titers were calculated at subject level from the quadruplicate tests of each serum sample and at group level. All hypothesis testing was done on log-transformed values. In addition, individual and collective level geometric mean titer ratios (GMTR) (T1/T0) were determined to quantify the increase in GM titers by vaccination. For each variable, the result was expressed as the point estimate with the 95% CI. Seroconversion was defined as a four-fold or more increase in GM titers between pre- and postvaccination samples (25).

Anti-MICA and anti-HLA Antibody Detection

Identification of serum IgG MICA Abs was performed using Luminex beads bound to single recombinant MICA antigens (Gen-Probe Transplant Diagnostics), as described previously (14). HLA class I and II antibody screening was performed according to the manufacturer's instructions using Luminex beads coated with HLA class I and HLA class II antigens (Gen-Probe Transplant Diagnostics), providing either a positive or negative result. Bound antibodies were identified using anti-human IgG coupled with phycoerythrin. A sample was considered positive for class I HLA antibodies if at least one of the seven class I HLA beads was positive (score 3) and positive for class II HLA antibodies if at least one of the 5 class II HLA beads was positive (score 3). *De novo* MICA or HLA sensitization was defined as the detection of MICA or HLA Abs at T1 that were absent at T0.

Statistical Analyses

The study was exploratory by design. We recruited as many patients as possible during the vaccination period, with no *a priori* sample size calculation or power. *Post hoc* analysis revealed that our study provided a 80% power to detect a reduction in the response rate to vaccination from 90% in controls to 60% in RT and HD patients with a type one error of 0.05. Summary statistics of antibody production are presented as geometric means with 95% CI. In spite of logarithmic transformation, the distribution of prevaccination titers was slightly right-skewed because of the large proportion of seronegative individuals. For this reason, between-group comparisons were done using the nonparametric Kruskal-Wallis method and the Wilcoxon rank-sum test to compare between-group differences. The increase in antibody titers after vaccination was tested with the paired Wilcoxon signed rank test. The effect of patient category on the postvaccination response was assessed by calculating the odds ratio of seroconversion. Between-group differences in the strength of the vaccination response were tested by the Kruskal-Wallis and Wilcoxon rank-sum tests. RT and HD patients differed markedly

Table 1. Population characteristics

Parameter	RT Patients ($n = 111$)	HD Patients ($n = 53$)	Controls ($n = 21$)	P
Males (%)	71 (64%)	33 (62%)	6 (29%)	0.09 0.8[a]
Age (years) (mean ± SD)	55 ± 12	64 ± 13	41 ± 12	<0.01 <0.01[a]
Diabetes (%)	36 (32%)	22 (42%)	NA	0.26
Months since RT or HD	34 (0.4 to 323)	32 (0.2 to 278)	NA	
Immunosuppression (%)			NA	
tacrolimus	58 (52%)	0		
cyclosporin	37 (33%)	1 (1.9%)		
mycophenolic acid	65 (59%)	0		
azathioprine	36 (32%)	0		
mTOR inhibitor	11 (10%)	0		
corticosteroids	91 (82%)	5 (9.4%)		
dose of methylprednisolone (mg/kg)[b]	0.05 (0.01 to 0.21)	0.09 (0.03 to 0.91)		

RT, renal transplant; HD, hemodialysis; NA, not applicable.
[a]Renal transplanted patients *versus* hemodialyzed patients.
[b]Median (minimum to maximum).

from controls in terms of age and gender. Possible confounding for these two covariates was investigated. We used the chi-squared test for gender and ANOVA for age. The odds ratio of seroconversion after adjustment for these patient characteristics was calculated by logistic regression modeling. The model was constructed to document lack of confounding by differences in patient characteristics without recurring to a specific algorithm of variable selection. Stepwise regression techniques were unsuitable because none of the covariates reached a sufficient degree of statistical significance to be included or retained in the model. The influence of patient category on the HLA and MICA immunization was assessed by comparing the proportions of patients that presented antibodies before and 1 month after vaccination, using the McNemar test. All of the tests were performed using Stata 10.1 software (StataCorp, College Station, TX). A two-tailed P value of less than 0.05 was used to reject the null hypothesis.

Results

Patients' Baseline Data

The characteristics of controls, HD patients, and RT patients are shown in Table 1. Women were more prevalent among controls, who were also younger than HD and RT patients. HD patients were significantly older than RT patients. More than 85% of the patients and controls were Caucasians, with the others being North and black Africans. Vaccination was performed at a median of 34 months after kidney transplantation and 32 months after the start of dialysis. Immunosuppression in RT patients consisted of a calcineurin inhibitor (tacrolimus, 52%; cyclosporine, 33%), a proliferation inhibitor (mycophenolic acid, 59%; azathioprine, 32%), or mTOR inhibitors (10%). Most of the patients were on methylprednisone (82%).

Serologic Vaccinal Responses

Anti-A/H1N1 Ab titers are shown in Table 2. There was no difference in GM titers before vaccination (T0) between the three groups. The GM titers differed significantly between the three groups at T1 ($P < 0.001$). Within each group, there was a significant increase in mean GM titers between T0 and T1 ($P < 0.001$). The ratio between the GM titers at T1 and T0 (GMTR) was highest in the controls, followed by dialysis and RT patients (38, nine, and five, respectively; $P < 0.001$). The GMTR was also significantly higher in HD as compared with RT recipients ($P = 0.02$) and higher in controls as compared with both RT and HD

Table 2. Anti-H1N1 antibodies

GMT (95% CI)	RT Patients ($n = 111$)	HD Patients ($n = 53$)	Controls ($n = 21$)	P
GMT day 0	11 (8 to 14)	8 (6 to 11)	9 (4 to 19)	0.56
GMT day 30	50 (34 to 72)	77 (44 to 136)	361 (166 to 782)	<0.001[a,b]
GMT ratio	5 (3 to 6)	9 (5 to 16)	38 (19 to 78)	<0.001[a,c]
Seroconversion (%)	49/111 (44%)	30/53 (57%)	19/21 (90%)	<0.001[a,d]

GM, geometric mean titer; CI, confidence interval; RT, renal transplant; HD, hemodialysis.
[a]$P < 0.001$ for intergroup comparisons.
[b]$P < 0.001$ for all intragroup comparisons between days 30 and 0.
[c]Post-tests: control *versus* HD, $P = 0.003$; control *versus* RT, $P = 0.001$; HD *versus* RT, $P = 0.02$.
[d]$P < 0.001$ by χ^2 for trend.

Table 3. Multivariate analysis of ESRD treatment modality on H1N1 vaccination response after adjustment for age and gender

Parameter	Odds Ratio	95% Confidence Interval	*P*
Hemodialysis *versus* control	0.14	0.03, 0.72	0.02
Transplantation *versus* control	0.09	0.02, 0.42	<0.01
Age (per 10 years)	1.05	0.80, 1.38	0.74
Male gender	0.94	0.50, 1.77	0.85

patients (Table 2). Moreover, seroconversion was more frequent among controls (90%) than among HD patients (57%) and RT patients (44%) ($P < 0.001$). There was a significant stepwise decrease in the proportion of responders between controls and HD and RT patients ($P < 0.001$, χ^2 for trend).

In the univariate analysis, the odds ratio (OR) of being a responder in HD and RT patients, respectively, was 0.13 (95% CI: 0.03 to 0.60, $P = 0.009$) and 0.08 (95% CI: 0.02 to 0.37, $P = 0.001$) as compared with controls. The multivariable logistic regression model showed that adjustment for age or gender did not change the odds of seroconversion in HD (adjusted OR: 0.14) and RT patients (adjusted OR: 0.09) (Table 3) as compared with controls. When RT patients were considered, univariate logistic regression model showed that age, gender, diabetes, level of renal function (as assessed by Modification of Diet in Renal Disease–estimated GFR), the type of immunosuppressive drug, and the time since transplantation were not associated with seroconversion rates (Table 4). Although RT patients treated with azathioprine and mycophenolic acid showed a similar proportion of seroconversion (50% *versus* 41%, $P = 0.41$), a significantly higher increase in antibody titers was observed in patients receiving azathioprine (GMTR, 7.7 *versus* 3.7 in patients receiving mycophenolic acid, $P = 0.03$).

Vaccination and MICA/HLA Immunization

The prevalence of MICA and HLA sensitization at baseline is shown in Table 5. The proportion of HLA-sensitized patients and controls did not change after vaccination, neither for class I nor for class II antibodies. Only two patients, one HD and one RT, developed *de novo* anti-HLA class II Abs. Along the same line, there was no significant change in the proportion of patients and controls with MICA Abs before and after vaccination. Of note, among the 16 RT patients sensitized against MICA before vaccination, only eight remained positive at T1, whereas four patients developed *de novo* MICA Abs.

Adverse Events

No serious event related to vaccination was reported during the study. Local inflammatory symptoms were infrequent and mild and resolved spontaneously without sequelae. No rejection episode was observed in RT recipients, and their kidney graft function remained stable during the 6 months after vaccination.

Discussion

The main data from our single-center investigation is that the rate of seroconversion to H1N1 vaccine was 90% in a control population, 57% in HD patients, and 44% in RT patients. Previous asymptomatic infection with the new influenza A/H1N1 2009 virus, as well as some minor degree of false-positive assays, might contribute to the

Table 4. Association between patient characteristics and response to H1N1 vaccination in renal transplant recipients

	Responders	% Responders	OR (95% CI)	*P*
Age at vaccination				
<55 years	27/54	50	1	
≥55 years	22/57	38.6	0.63 (0.30 to 1.34)	0.23
Gender				
female	21/40	52.5	1	
male	28/71	39.4	0.59 (0.27 to 1.29)	0.18
Diabetes				
no	37/75	49.3	1	
yes	12/36	33.3	0.51 (0.22 to 1.17)	0.12
Renal function (MDRD) (ml/min per 1.73 m^2)				
>60	16/34	47.1	1	
30 to 59	27/60	45	0.92 (0.40 to 2.14)	0.85
15 to 29	6/17	35.3	0.61 (0.18 to 2.04)	0.43
Immunosuppression				
cyclosporine	16/37	43.2	1	
tacrolimus	24/58	41.4	0.93 (0.40 to 2.13)	0.86
azathioprine	18/36	50	1	
mycophenolic acid	27/65	41.5	0.71 (0.31 to 1.61)	0.41
Years since transplantation				
<1	13/37	35.1	1	
1 to 5	20/38	52.6	2.1 (0.8 to 5.2)	0.13
>5	16/36	44.4	1.5 (0.6 to 3.8)	0.42

OR, odds ratio; CI, confidence interval; MDRD, Modification of Diet in Renal Disease.

Table 5. HLA and MICA immunization prevalence before (T0) and after (T1) H1N1 vaccination among renal transplanted patients and hemodialysis patients

Proportion of Immunized Patients (%)	RT Patients ($n = 111$)		HD Patients ($n = 53$)		Controls ($n = 21$)		P
	T0	T1	T0	T1	T0	T1	
anti-HLA Cl I	15%	14%	25%	25%	19%	19%	1
anti-HLA Cl II	14%	14%	23%	25%	10%	10%	1
anti-MICA	14%	11%	15%	19%	5%	10%	0.39, 0.48, 1[a]

HLA, human leukocyte antibody; Cl, class; MICA, MHC class I-related chain A antibody; RT, renal transplanted patients; HD, hemodialysis patients.
[a]Before *versus* after vaccination, in renal transplant and hemodialysis patients and controls, respectively.

presence at baseline of low titers in a small proportion of patients (4). Our seroconversion rates are similar to those reported with the seasonal influenza vaccine both in RT (8–11,26,27) and HD (12,28) patients. As compared with seroconversion rates recorded previously with seasonal H1N1 vaccines, such as A/New Caledonia/20/99 (H1N1), the proportion of responders among our RT patients seems to be higher. Indeed, seroconversion was only 10% in a series of 66 RT patients (*versus* 58% in healthy controls, $P < 0.001$) in a French study (8) and 36% in a Brazilian uncontrolled study of 69 patients (11). However, one should keep in mind that with seasonal vaccines, people are likely to have been vaccinated previously and therefore may show less robust subsequent vaccine response. Since the submission of our work, another group reported that the proportion of immune response after a single dose of AS03-adjuvanted H1N1 vaccine was 35% among adult RT patients, close to the 44% reported here (29). In pediatric RT patients, a single injection of a MF59-adjuvanted H1N1 vaccine (similar to the ASO3 adjuvant) resulted in a 19% rate of seroconversion (30). Of note, these studies assayed seroconversion by an hemagglutination inhibition test, whereas we performed a seroneutralization assay. This latter test is likely to better predict the ability of the patient serum to protect against H1N1 infection.

Two studies published after submission of our work show a similar 60% seroconversion rate after H1N1 vaccination among HD patients (31,32), but they did not report on a direct comparison between HD and RT patients as we did here. In spite of the older age of HD patients, their serologic response was stronger than in our RT cohort with regard to both the proportion of responders and their titers against H1N1. This supports the recommendations that vaccinations should be performed as early as possible in the course of renal diseases (2,33). Among RT patients, those receiving mycophenolic acid had a lower increase in Ab response than those receiving azathioprine. This observation is similar to previous reports pertaining to influenza vaccination (9,11,26). This work brings additional data supporting the safety of influenza vaccination in renal transplant recipients (8,33) and also documents that vaccination does not induce sensitization against HLA or MICA in dialyzed patients, who can become candidates for kidney transplantation. *De novo* HLA sensitization was recorded in only two patients (1.1%), a proportion even lower than the 4.8% reported by Candon et *al*. (8). Our findings are in line with other recent publications that did not report an increase in acute rejection rates triggered by vaccination (8,9,34). Similar to HLA sensitization, the prevalence of MICA Abs remained stable after vaccination, and the frequency of *de novo* MICA sensitization was lower than 5%. Nevertheless, a recent report indicates that influenza vaccination can trigger allo-reactive T cells among both controls and RT patients (34). This supports the common practice to perform vaccination beyond 3 months of transplantation to allow the restoration of an effective antiviral immune response while at the same time minimizing the risk of acute rejection.

We must acknowledge that our study has a number of limitations. First, the sample size of our cohort of RT and HD patients was underpowered to allow for robust subgroup analysis. Second, we did not compare different vaccine schemes. Third, we did not compare different vaccines.

Nevertheless, our data show that the influenza A/H1N1-adjuvanted vaccine is safe, although the seroconversion rate is only approximately 50% in HD and RT patients. Further trials with higher vaccine doses and/or a booster may be warranted in these populations (9,35).

Acknowledgments

The authors thank B. Borre, N. Lietaer, I. Moerman, C. Addino, and I. Goncalves for their expert help with patient vaccination and serum sample harvesting.

The study was funded by the research funds of the Renal Transplant Clinic.

Disclosures

None.

References

1. Kumar D, Morris MI, Kotton CN, Fischer SA, Michaels MG, Allen U, Blumberg EA, Green M, Humar A, Ison MG: Guidance on novel influenza A/H1N1 in solid organ transplant recipients. *Am J Transplant* 10: 18–25, 2010
2. Avery RK, Michaels M: Update on immunizations in solid organ transplant recipients: What clinicians need to know. *Am J Transplant* 8: 9–14, 2008
3. 2009 pandemic influenza A (H1N1) virus infections - Chicago, Illinois, April-July 2009. *MMWR Morb Mortal Wkly Rep* 58: 913–918, 2009
4. Hancock K, Veguilla V, Lu X, Zhong W, Butler EN, Sun H, Liu F, Dong L, DeVos JR, Gargiullo PM, Brammer TL, Cox

NJ, Tumpey TM, Katz JM: Cross-reactive antibody responses to the 2009 pandemic H1N1 influenza virus. *N Engl J Med* 361: 1945–1952, 2009
5. Use of influenza A (H1N1) 2009 monovalent vaccine: Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. *MMWR Recomm Rep* 58: 1–8, 2009
6. Clark TW, Pareek M, Hoschler K, Dillon H, Nicholson KG, Groth N, Stephenson L: Trial of 2009 influenza A (H1N1) monovalent MF59-adjuvanted vaccine. *N Engl J Med* 361: 2424–2435, 2009
7. Greenberg ME, Lai MH, Hartel GF, Wichems CH, Gittleson C, Bennet J, Dawson G, Hu W, Leggio C, Washington D, Basser RL: Response to a monovalent 2009 influenza A (H1N1) vaccine. *N Engl J Med* 361: 2405–2413, 2009
8. Candon S, Thervet E, Lebon P, Suberbielle C, Zuber J, Lima C, Charron D, Legendre C, Chatenoud L: Humoral and cellular immune responses after influenza vaccination in kidney transplant recipients. *Am J Transplant* 9: 2346–2354, 2009
9. Scharpe J, Evenepoel P, Maes B, Bammens B, Claes K, Osterhaus AD, Vanrenterghem Y, Peetermans WE: Influenza vaccination is efficacious and safe in renal transplant recipients. *Am J Transplant* 8: 332–337, 2008
10. Birdwell KA, Ikizler MR, Sannella EC, Wang L, Byrne DW, Ikizler TA, Wright PF: Decreased antibody response to influenza vaccination in kidney transplant recipients: A prospective cohort study. *Am J Kidney Dis* 54: 112–121, 2009
11. Salles MJ, Sens YA, Boas LS, Machado CM: Influenza virus vaccination in kidney transplant recipients: Serum antibody response to different immunosuppressive drugs. *Clin Transplant* 24: E17–E23, 2010
12. Song JY, Cheong HJ, Ha SH, Kee SY, Jeong HW, Kim WJ: Active influenza immunization in hemodialysis patients: Comparison between single-dose and booster vaccination. *Am J Nephrol* 26: 206–211, 2006
13. Société de Néphrologie. Grippe A (H1N1): Propositions de recommendations pour les patients transplantés. ICAR, editor. 12–10-2009. http://www.soc-nephrologie.org/PDF/enephro/recommandations/grippe/SN_SFD_SFT.pdf
14. Lemy A, Andrien M, Wissing KM, Ryhahi K, Vandersarren A, Racape J, Heylen C, Ghisdal L, Broeders E, Vereerstraeten P, Toungouz M, Abramowicz D: Major histocompatibility complex class 1 chain-related antigen a antibodies: Sensitizing events and impact on renal graft outcomes. *Transplantation* 90: 168–174, 2010
15. Zou Y, Stastny P, Susal C, Dohler B, Opelz G: Antibodies against MICA antigens and kidney-transplant rejection. *N Engl J Med* 357: 1293–1300, 2007
16. Terasaki PI, Ozawa M, Castro R: Four-year follow-up of a prospective trial of HLA and MICA antibodies on kidney graft survival. *Am J Transplant* 7: 408–415, 2007
17. Morel S, Didierlaurent A, Bourguignon P, Delhaye S, Baras B, Jacob V, Planty C, Elouahabi A, Harvengt P, Carlsen H, Kielland A, Chomez P, Garcon N, Van Mechelen M: Adjuvant system AS3 containing alpha-tocopherol modulates innate immune response and leads to improved adaptive immunity. *Vaccine* 29: 2461–2473, 2011
18. Langley JM, Risi G, Caldwell M, Gilderman L, Berwald B, Fogarty C, Poling T, Riff D, Baron M, Frenette L, Sheldon E, Collins H, Shepard M, Dionne M, Brune D, Ferguson L, Vaughn D, Li P, Fries L: Dose-Sparing H5N1 A/Indonesia/05/2005 Pre-pandemic Influenza Vaccine in Adults and Elderly Adults: A phase III, placebo-controlled, randomized study. *J Infect Dis* 203: 1729–1738, 2011
19. Mizutani K, Terasaki P, Rosen A, Esquenazi V, Miller J, Shih RN, Pei R, Ozawa M, Lee J: Serial ten-year follow-up of HLA and MICA antibody production prior to kidney graft failure. *Am J Transplant* 5: 2265–2272, 2005
20. Lee PC, Zhu L, Terasaki PI, Everly MJ: HLA-specific antibodies developed in the first year posttransplant are predictive of chronic rejection and renal graft loss. *Transplantation* 88: 568–574, 2009
21. Lachmann N, Terasaki PI, Budde K, Liefeldt L, Kahl A, Reinke P, Pratschke J, Rudolph B, Schmidt D, Salama A, Schonemann C: Anti-human leukocyte antigen and donor-specific antibodies detected by luminex posttransplant serve as biomarkers for chronic rejection of renal allografts. *Transplantation* 87: 1505–1513, 2009
22. Rowe T, Abernathy RA, Hu-Primmer J, Thompson WW, Lu X, Lim W, Fukuda K, Cox NJ, Katz J: Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. *J Clin Microbiol* 37: 937–943, 1999
23. Perkins FT: A ready reckoner for the calculation of geometric mean antibody titres. *J Gen Microbiol* 19: 540–541, 1958
24. Horne AD: The statistical analysis of immunogenicity data in vaccine trials. *Ann N Y Acad Sciences* 754: 329–346, 1995
25. The European Agency for the evaluation of Medicinal Products (EMEA) and committee for proprietary medicinal products (CPMP): Note for guidance on harmonisation of requirements for influenza vaccines. CPMP/BWP/214/96; 12 March 1997 http://www.emea.eu.int/pdfs/human/bwp/021496en.pdf, 2007
26. Smith KG, Isbel NM, Catton MG, Leydon JA, Becker GJ, Walker RG: Suppression of the humoral immune response by mycophenolate mofetil. *Nephrol Dial Transplant* 13: 160–164, 1998
27. Sanchez-Fructuoso AI, Prats D, Naranjo P, Fernandez-Perez C, Gonzalez MJ, Mariano A, Gonzalez J, Figueredo MA, Martin JM, Paniagua V, Fereres J, Gomez de la Concha E, Barrientos A: Influenza virus immunization effectivity in kidney transplant patients subjected to two different triple-drug therapy immunosuppression protocols: Mycophenolate versus azathioprine. *Transplantation* 69: 436–439, 2000
28. Scharpe J, Peetermans WE, Vanwalleghem J, Maes B, Bammens B, Claes K, Osterhaus AD, Vanrenterghem Y, Evenepoel P: Immunogenicity of a standard trivalent influenza vaccine in patients on long-term hemodialysis: An open-label trial. *Am J Kidney Dis* 54: 77–85, 2009
29. Brakemeier S, Schweiger B, Lachmann N, Glander P, Schonemann C, Diekmann F, Neumayer HH, Budde K: Immune response to an adjuvanted influenza A H1N1 vaccine (Pandemrix®) in renal transplant recipients. *Nephrol Dial Transplant* 2011, first published online May 25, 2011. doi: 10.1093/ndt/gfr 278 in press
30. Esposito S, Meregalli E, Daleno C, Ghio L, Tagliabue C, Valzano A, Serra D, Galeone C, Edefonti A, Principi N: 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. *Nephrol Dial Transplant* 26: 2018–2024, 2011
31. Labriola L, Hombrouck A, Marechal C, Van GS, Brochier B, Thomas I, Jadoul M, Goubau P: Immunogenicity of an adjuvanted 2009 pandemic influenza A (H1N1) vaccine in haemodialysed patients. *Nephrol Dial Transplant* 26: 1424–1428, 2011
32. Dikow R, Eckerle I, Ksoll-Rudek D, Hampel H, Schwenger V, Zeier M, Schnitzler P, Sommerer C: Immunogenicity and efficacy in haemodialysis patients of an AS03(A)-adjuvanted vaccine for 2009 pandemic influenza A (H1N1): A nonrandomized trail. *Am J Kidney Dis* 57: 716–723, 2011
33. Guidelines for vaccination of solid organ transplant candidates and recipients. *Am J Transplant.* 4[Suppl 10]: 160–163, 2004
34. Danziger-Isakov L, Cherkassky L, Siegel H, McManamon M, Kramer K, Budev M, Sawinski D, Augustine JJ, Hricik DE, Fairchild R, Heeger PS, Poggio ED: Effects of influenza immunization on humoral and cellular alloreactivity in humans. *Transplantation* 89: 838–844, 2010
35. Blumberg EA, Albano C, Pruett T, Isaacs R, Fitzpatrick J, Bergin J, Crump C, Hayden FG: The immunogenicity of influenza virus vaccine in solid organ transplant recipients. *Clin Infect Dis* 22: 295–302, 1996

Received: May 17, 2011 **Accepted:** August 17, 2011

Published online ahead of print. Publication date available at www.cjasn.org.