Influence of renal replacement therapy on immune response after one and two doses of the A(H1N1) pdm09 vaccine

Luis F. Quintana[a], Nuria Serra[a], Patricia De Molina-Llauradó[b,c], Miquel Blasco[a], Mikel Martinez[b,c], Begoña Campos[d], Jose M. Bayas[e], Tomás Pumarola[b,c], Josep M. Campistol[a]

[a]Department of Nephrology and Renal Transplantation, Hospital Clinic de Barcelona, IDIBAPS, University of Barcelona, Barcelona, Spain. [b]Department of Clinical Microbiology, Hospital Clinic de Barcelona, IDIBAPS, University of Barcelona, Barcelona, Spain. [c]Barcelona Centre for International Health Research (CRESIB), Hospital Clinic de Barcelona, IDIBAPS, University of Barcelona, Barcelona, Spain. [d]Biostatistics Unit, Department of Public Health, Hospital Clinic de Barcelona, IDIBAPS, University of Barcelona, Barcelona, Spain. [e]Preventive Medicine and Epidemiology Unit Hospital Clínic de Barcelona, IDIBAPS, University de Barcelona, Barcelona, Spain.
Correspondence: Luis F. Quintana, Servicio de Nefrología y Trasplante renal, Hospital Clinic, Barcelona, Spain. E-mail: lfquinta@clinic.ub.es

Background Patients with end-stage renal disease have a reduced response to vaccination because of the general suppression of the immune system associated with uraemia.

Objectives We evaluated the immune response and differential factors in the immunogenecity to an adjuvanted A(H1N1) pdm09 vaccine (Pandemrix®) in four populations of renal patients after one and two doses of vaccine.

Patients Methods 151 patients were included in this study: 58 chronic haemodialysis patients, 52 renal allograft recipients, 14 peritoneal dialysis patients and 27 patients with advanced chronic kidney disease in preparation for kidney replacement therapy. Influenza-specific antibody levels were measured by monitoring A(H1N1) pdm09 titres using a haemagglutination inhibition assay.

Results The seroconversion rate at 42 days after two vaccine doses was 80% in the haemodialysis group, 64·9% in the renal allograft recipients group, 100% in the advanced chronic kidney disease group and 71·4% in the peritoneal dialysis group ($P = 0·041$).

Conclusions Immune response to two doses of the influenza A H1N1 vaccine is dissimilar in the four renal conditions, confirming that seroprotection in pre-dialysis, haemodialysis and peritoneal dialysis is similar to that in the general population vaccinated with one dose. In contrast, renal transplant recipients with good allograft function showed inadequate protection and triple immunosuppressive therapy including calcineurin inhibitors, mycophenolate and steroids negatively influenced seroconversion after vaccination in renal recipients.

Keywords A(H1N1) pdm09, dialysis kidney disease, renal transplantation, Vaccine

Introduction

Prevention of infection is essential to increase the life expectancy of patients with renal disease. Infection in these patients leads to high morbidity and mortality, and antimicrobial therapy is often less effective than in immunocompetent hosts.[1] Patients with end-stage renal disease (ESRD) have a reduced response to vaccination because of the general suppression of the immune system associated with uraemia. Compared with vaccination in patients without ESRD, for example, dialysis patients have a lower antibody titre and an inability to maintain adequate antibody titres over time.[2,3] The relatively low antibody response to vaccines also appears to correlate with the degree of renal failure, but not with the specific mode of dialysis.[4]

The altered acquired immunity in patients with ESRD may be caused by disturbances in T lymphocytes and antigen-presenting cells, but additional studies are required.[5–7] Little information has been published on the effects of dialysis adequacy on the antibody response to vaccination. There is, however, indirect evidence that increasing dialysis may be associated with an enhanced response. In a study of 32 nutritionally replete peritoneal dialysis patients immunised with the hepatitis B vaccine, the initial weekly Kt/V was 2·37 and 2·01 in converters and non-converters, respectively.[8]

Apparently, renal transplant recipients respond to vaccines similarly to chronic dialysis patients: the antibody response is often less than in patients without nephropathy and protective antibodies fall rapidly. Despite the evidence of decreased efficacy, current recommendations are to vaccinate patients with ESRD.[9,10] Although immunizations are important to prevent infection, many immunocompromised patients are unable to mount protective immune responses and live vaccines are usually avoided.[11]

During the 2009–2010 influenza season, a monovalent vaccine was needed to vaccinate individuals against A (H1N1) pdm09, because the trivalent (seasonal) influenza vaccine did not contain antigens from this strain. When the vaccine supply was limited in the autumn of 2009, health officials created a priority list for administration of A(H1N1) pdm09 vaccine that included, amongst others, individuals with chronic renal disease because they have an increased risk of influenza complications.[12]

In the present study, we evaluated the immune response to an adjuvanted A(H1N1) pdm09 vaccine (Pandemrix®) in four populations of renal patients (haemodialysis, renal transplantation, peritoneal dialysis and pre-dialysis) after one and two doses of vaccination by monitoring A(H1N1) pdm09 titres. We also aimed to evaluate the influence of age, renal function, time on haemodialysis, time since the kidney transplantation, diabetes mellitus, haemoglobin levels, parathyroid hormone (PTH) levels, dialysis dose and the immunosuppressive therapy as differential factors in the immunogenecity of the vaccine amongst patients receiving these four types of renal replacement therapy.

Material and methods

Study population

From November to December of 2009, a total of 151 patients were included in this study: 58 chronic haemodialysis patients, 52 renal allograft recipients, 14 peritoneal dialysis patients and 27 patients with advanced chronic kidney disease in preparation for kidney replacement therapy (GRFe MDRD-4) lower than 20 ml/min).

Haemodialysis patients were from our in-hospital unit, and all were receiving high-flux haemodialysis and had optimum dialysis parameters (Kt > 45 l).

Renal allograft recipients had been transplanted at least 6 months before the beginning of the study and had good renal function (GRFe MDRD-4 > 50 ml/min). All the patients had previously received the seasonal vaccination (approximately 1 month before). The main demographic, clinical and laboratory data were obtained from clinical records and are shown in Table 1. There were not subjects under treatment with steroids in non-transplant patients (pre-dialysis and dialysis groups). This study was

Table 1. Demographics and laboratory data

	Renal Transplant	Haemodialysis	Peritoneal Dialysis	Pre-dialysis
Age (years)	54·04 ± 12·04	59·84 ± 15	55·29 ± 14·72	70·59 ± 10·1
Gender (male/female)	26/26	34/24	9/5	16/11
Time on dialysis (months)		45·91 ± 56·48	25 ± 13·27	
Time after transplantation (months)	82·58 ± 87·36			
Creatinine pre-vaccine (mg/dl)	1·44 ± 0·29			2·78 ± 0·82
GFR MDRD-4 pre-vaccine (ml/min/1·73 m²)	52·42 ± 10·2			22·21 ± 7·27
Diabetes mellitus (%)	17·3	15·5	7·1	25·9
Serum proteins (g/l)	6·91 ± 0·56	6·80 ± 0·59	6·80 ± 0·49	7·21 ± 0·51
Haemoglobin (g/dl)	12·65 ± 1·77	11·42 ± 1·52	11·95 ± 2·31	12·18 ± 1·57
Leucocytes 10^3/mm³	7270 ± 2650	7230 ± 2110	7720 ± 2540	7370 ± 2400
PTH (pg/ml)	239·06 ± 146·76	278·58 ± 216·62	381·93 ± 332·94	259·46 ± 171·86
Kt (*l*)		69·87 ± 20·44		
Kt/V			1·93 ± 0·2	
Tacrolimus + MMF + steroids treatment	17 (32·7%)			
Sirolimus + MMF treatment	6 (11·5%)			
Tacrolimus + MMF treatment	7 (13·5%)			
Monotherapy (tacrolimus or sirolimus)	8 (15·4%)			
Other immunosuppressive treatment	14 (26·9%)			

GFR, glomerular filtration rate; MDRD-4, Modification of Diet in Renal Disease; PTH, parathyroid hormone; MMF, mycophenolate mofetil.

approved by our internal ethics review board. All patients involved in the study signed the informed consent form accepted by the internal board at our centre for the antibody test.

Vaccine

All patients involved in the study received two 3·75 μg doses of adjuvant – containing A/California/7/2009 (H1N1) v-like strain vaccine (Pandemrix®) on day 0 and after 21 days. The vaccine was administered into the deltoid muscle. Information on local and general symptoms was recorded by each subject using diary cards for the first 7 days following each vaccination. Furthermore, clinical follow-up was performed during 6 months after the vaccination.

Sample sera

Three sample sera were obtained from each individual, once before vaccination (time A), again at 21 days (time B: just before administration of the second dose) and at 42 days (time C: 21 days after the second dose of the vaccine) after the first dose. Sera were stored at −80°C until antibodies against A(H1N1) pdm09 were determined.

Antibody determination

Influenza-specific antibody levels were measured using a haemagglutination inhibition (HI) assay with chicken red blood cells (RBC) according to the standardized protocol of the World Health Organisation (WHO).[12] In brief, serum non-specific inhibitors were removed with receptor-destroying enzyme treatment overnight at 37°C, followed by inactivation at 56°C for 30 min. The standard antigen was diluted to contain four haemagglutinin units, and back titration was performed. An RDE-treated serum was two-fold serially diluted in v-bottom microtitre plates. Then, diluted sera were mixed with 25 μl of H1N1pdm antigen (2010–2011 WHO influenza reagent kit for the identification of influenza isolates).

After 1 h of incubation at room temperature, 50 μl of RBC [diluted 0·05% in phosphate buffered saline (PBS)] was added to the wells. Positive and negative serum controls were included for each plate. Titres were expressed as the reciprocal of the highest dilution of serum that inhibited haemagglutination.

The following serological parameters were evaluated:
1. Geometric mean titres (GMT) of HI.
2. The seroconversion rate was defined as the rate of patients with a four-fold increase in antibody titres against influenza A H1N1 after vaccination. Seroconversion factor was defined as the level of increase in GMT antibody titres before and after vaccination. The seroprotection rate was defined as the percentage of patients with an antibody titre of ≥1/40.[13]

Statistical analysis

Student's t-test was used to compare continuous parameters, and the Fisher's exact test or chi-square test for trend was used for categorical data. All P-values were obtained as two sided and were considered to be significant if <0·05. Logistic regression was used to analyse the association of clinical outcome of a protective immune response with the following parameters: age, dose of immunosuppressive agent gender, time since transplantation and panel rate antibody PRA before vaccination. Only variables with a P-value <0·15 were retained in the final model. All analyses were performed using PASW Statistics 18 software (SPSS).

Results

Of the 151 patients included in the study, 33 (14 under haemodialysis, 5 renal transplant recipients, 6 under peritoneal dialysis and 8 with ESRD) showed seroprotection against A(H1N1) pdm09 in the pre-vaccination sample. The main demographic and clinical data of the patients involved in this study and their immunosuppressive therapy are given in Table 1. There were no statistically significant differences between the four groups in age, gender, prevalence of HIV infection, prevalence of diabetes mellitus or cause of renal disease.

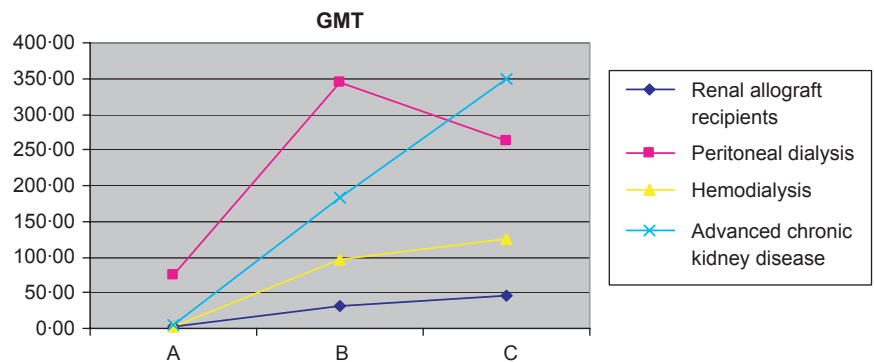Geometric mean titres (GMT) of haemagglutination inhibition at Times A, B and C are shown in Figure 1. The

Figure 1. Geometric mean titres (GMT) of haemagglutination inhibition at Times A, B and C.

overall vaccine seroconversion rate at 21 days after the first vaccine dose (time B) was 69·8%. By groups, the seroconversion rate at this time was 79·6% in the haemodialysis group, 55·8% in the renal allograft recipient group, 80% in the advanced chronic kidney disease group and 55·6% in the peritoneal dialysis group ($P = 0.04$).

At 42 days after vaccination with the first vaccine dose and 21 days after the second dose (time C), the overall seroconversion rate was higher than after the first dose (time B) in 77·1%. By groups, the seroconversion rate at 42 days after two vaccine doses was 80% in the haemodialysis group, 64·9% in the renal allograft recipients group, 100% in the advanced chronic kidney disease group and 71·4% in the peritoneal dialysis group ($P = 0.041$). Table 2 shows the seroprotection rates at times B and C and the seroconversion factor. At 42 days after two vaccine doses, the seroconversion and seroprotection rates were significantly lower in the transplant recipient group ($P < 0.05$).

Age, gender, cause of renal disease, diabetes mellitus prevalence, serum proteins, haemoglobin level, PTH and HIV infection showed no influence on the seroconversion rate after vaccination amongst the four groups of renal patients.

In renal transplant recipients, no influence was found for time since transplantation, proteinuria, serum proteins, haemoglobin level or PTH, but immunosuppressive therapy significantly influenced the efficacy of the vaccine.

Seroconversion rates were 35·3% (6/17) in renal transplant patients using a triple therapy including a calcineurin inhibitor (CNI), mycophenolate mofetil (MMF) and ste-roids, 83·3% (*n*:5/6) in patients in double therapy with MMF plus a *mammalian target of rapamycin* (M-TOR) inhibitor, 71·4% (5/7) in patients with double therapy with MMF + CNI and 87·5%(7/8) in patients under monotherapy with CNI or an M-TOR inhibitor.

There was a statistically significant difference amongst the four immunosuppressive treatment groups ($P = 0.02$) using a chi-square test for trend, with the lowest seroconversion rate in patients with triple therapy comparing with those on double and monotherapy. Equally, no significant differences in the efficacy of the vaccine were found in patients with double therapy receiving MMF + MTOR inhibitor vs MMF + CNI or CNI versus M-TOR inhibitor monotherapy.

A significant difference in the seroconversion rate was observed depending on whether patients were on steroid therapy at each point in this study; 94·1% (16/17) of patients without steroid therapy achieved seroprotection after this vaccination protocol, whilst only 47·3% (9/19) of the patients under steroid therapy achieved an antibody titre of ≥1/40 ($P = 0.003$ at times B and C).

Therapy with MMF or an M-TOR inhibitor *per se* had no statistically significant influence on the seroprotection rate.

The incidence of adverse events (local and general symptoms) at vaccination is shown in Table 3. No severe adverse events were detected during the follow-up (6 months after the vaccination with the first vaccine dose). In the transplant group, no episodes of acute graft rejection have been registered.

Table 2. Seroconversion rate, seroprotection rate and seroconversion factor

	Seroconversion Day 21	Seroconversion Day 42	Seroprotection Day 21 (%)	Seroprotection Day 42 (%)	Seroconversion Factor Day 42
Haemodialysis	79·6	80	74·1	80	35·5
Renal Transplant	55·8	64·9	62·8	67·7	19·2
Pre-dialysis	80	100	85	100	60·1
Peritoneal Dialysis	55·6	71·4	88·9	100	3·4
P-value	0·04	0·041	0·17	0·02	0·04

Table 3. Local and general symptoms rates at days 21 and 42

	Local symptoms Day 21 (%)	Local symptoms Day 42 (%)	General symptoms Day 21 (%)	General symptoms Day 42 (%)
Renal Transplant	61·51	30·76	40·38	15·38
Haemodialysis	15·51	0	10·34	0
Peritoneal Dialysis	57·14	25·57	35·71	35·71
Pre-dialysis	37	11·1	18·51	18·51

Discussion

Based on previous experience that, like many immunocompromised patients, renal patients are unable to mount protective immune responses after vaccination, we administered two doses of the A (H1N1) pdm09 vaccine to this cohort of renal patients during the 2009–2010 influenza season.

Previous studies showed that the response to a single dose of the A (H1N1) pdm09 vaccine was suboptimal in renal transplant recipients, and a recent report has demonstrated the lack of efficacy of this schedule in a group of haemodialysis patients.[14,15] To our knowledge, this is the first study that evaluates the efficacy of this vaccine in four groups of renal patients and using two doses.

The present study showed that seroprotection before vaccination differed widely amongst these four groups of renal patients, demonstrating pre-vaccination titres of >1/40 in 30% of the pre-dialysis patients, 60% of the peritoneal dialysis patients, 25% of the haemodialysis patients and 13·5% of the renal transplant recipients. Although this seroprotection rate was lower than that in healthy population,[16] these results demonstrate a highly dissimilar pre-existing level of immunity in the four groups of renal patients before vaccination and suggest that the response to A(H1N1) pdm09 could be influenced by the differential immunological status amongst these four types of renal replacement therapy. Notably, in this study, the overall rate of seroconversion and seroprotection in pre-dialysis, haemodialysis and peritoneal dialysis patients after two vaccine doses reached the targets reported in the literature for an optimal response after one dose of the seasonal vaccination in healthy subjects or with controlled chronic illness. In contrast, the seroprotection rate after two doses of the A(H1N1) pdm09 vaccine was sub-optimal in renal transplant recipients showing a pre-vaccination titre <1/40.

Compared with vaccination in dialysis patients, renal recipients had a lower antibody titre and an inability to maintain adequate antibody titres over time. Indeed, in both the pre-dialysis and peritoneal dialysis groups, the seroprotection rate after two vaccine doses was 100%, showing that the vaccine efficacy in these groups of renal patients was similar to that in the general population vaccinated with a single dose.[16] The seroconversion rate in the peritoneal dialysis population was lower because 6 of the 14 patients in this group had a pre-existing seroprotection level of >1/40 before vaccination.

There is little information on the effects of dialysis dose on seroconversion after vaccination. However, apart from the use of two vaccine doses, a better and more homogeneous antibody response after vaccination in these haemodialysis patients could be related to the fact that they received high-flux haemodialysis and had optimum dialysis adequacy (Kt >45 l). Indeed, Crespo et al.[15] recently reported that the seroconversion rate after one dose of the vaccine was only 33% in haemodialysis patients receiving a lower dialysis dose (Kt/V single pool 1·8).

Renal transplantation is the standard of care for patients with ESRD. Recent improvements in kidney transplantation have been driven largely by lower acute rejection rates and better long-term graft survival attributed to immunosuppressive agents. Standard immunosuppressive protocols to prevent acute graft rejection in this setting involve three major groups of drugs: CNI, anti-metabolites and steroids. There is no doubt that corticosteroids and CNI are the cornerstone of these therapeutic protocols and have played an important role in improving graft survival, mainly because of the reduction of acute rejection episodes.[17] In transplant recipients, influenza can range from a mild illness to severe lower respiratory tract disease, and immunization of target groups was recommended as an effective way to reduce the impact of the 2009–2010 influenza pandemic.[18] Analysis of the data reported in this work supports previous observations indicating that the level of immunosuppression seems to be an important variable determining the antibody response to a vaccine. Salles et al.[19] showed that the use of MMF decreased the immune response to seasonal influenza vaccine, although other studies showed no influence of the type of immunosuppressive therapy on seroconversion rate after A(H1N1) pdm09 vaccine.[14,15] A striking finding was that, in this cohort, immunosuppression consisting of triple therapy with MMF + CNI+ steroids had a deleterious effect on the antibody response to the vaccine, even when two vaccine doses were used in these recipients; this negative impact was more marked when we analysed the isolated influence of steroid therapy on the immunogenicity to this vaccination protocol.

In general, for influenza vaccination in other conditions, studies suggest that immunosuppressive medications may partially dampen the immune response, particularly when multiple immunosuppressive medications are used.[20] This work suggests that strong immunosuppression may be responsible for a lower response to the vaccine in transplant recipients and looks like a dose–response effect as the number of agents rises from mono to triple therapy, but additional studies are required to confirm this possibility.

With seasonal influenza vaccine, there has been anecdotal concern for vaccine-triggered allograft rejection.[21,22] However, immunogenicity and safety studies of influenza vaccine in transplant patients to date have not shown an increased risk of rejection, and epidemiologic data have not supported an association between vaccination and allograft rejection. In this study, vaccination with Pandemrix® was well tolerated, only a few mild reactions occurred, and there were no episodes of acute graft rejection. However, experience with the safety of novel vaccine adjuvants in

transplantation is limited and should be analysed in larger and prospective studies.

Pandemrix is combined with the proprietary ASO3 adjuvant and designed specifically to induce an increased antibody response. Traditional influenza A vaccine is not adjuvanted, and the qualitative effects of the adjuvant on the immune response may be important in relation to the effect of immunosuppressive drugs on the antibody response. The paper by Salles [19] used seasonal vaccine that does not contain an adjuvant, whilst the work of Labriola [14] and Crespo [15] used Pandemrix. Although in both cases, transplant patients responded less well than dialysis patients when vaccinated, certain formulations may work better in transplant patients than others and could be investigated in the near future.

These data question the efficacy of the A(H1N1) pdm09 vaccine in transplant patients under triple immunosuppression therapy and demonstrate the need for new studies with a larger population of renal transplant recipients to evaluate the efficacy and safety profile of the vaccine in these patients before issuing a general recommendation.

In conclusion, the results reported herein show that immune response to one and two doses of the A(H1N1) pdm09 vaccine is dissimilar in the four renal replacement therapy conditions, confirming that seroprotection in predialysis, haemodialysis and peritoneal dialysis is similar to that in the general population vaccinated with one dose. These data corroborate that, as reported in children under 12 years of age,[23] ESRD and dialysis patients require two doses for adequate protection.

In contrast, renal transplant recipients with good allograft function and under heavy immunosuppression showed inadequate protection after two doses of the vaccine and triple immunosuppressive therapy including calcineurin inhibitors (ACI), MMF and steroids negatively influenced seroconversion after vaccination in renal recipients. In particular, steroid therapy was of importance in this cohort.

References

1 Hibberd PL, Rubin RH. Approach to immunization in the immunosuppressed host. Infect Dis Clin North Am 1990; 4:123–142.
2 Rodby RA, Trenholme GM. Vaccination of the dialysis patient. Semin Dial 1991; 4:102.
3 Dinits-Pensy M, Forrest GN, Cross AS, Hise MK. The use of vaccines in adult patients with renal disease. Am J Kidney Dis 2005; 46:997.
4 Kausz A, Pahari D. The value of vaccination in chronic kidney disease. Semin Dial 2004; 17:9.
5 Eleftheriadis T, Antoniadi G, Liakopoulos V et al. Disturbances of acquired immunity in hemodialysis patients. Semin Dial 2007; 20:440.
6 Litjens NH, Huisman M, van den Dorpel M, Betjes MG. Impaired immune responses and antigen-specific memory CD4 + T cells in hemodialysis patients. J Am Soc Nephrol 2008; 19:1483.
7 Agrawal S, Gollapudi P, Elahimehr R et al. Effects of end-stage renal disease and haemodialysis on dendritic cell subsets and basal and LPS-stimulated cytokine production. Nephrol Dial Transplant 2010; 25:737.
8 Dacko C, Holley JL. The influence of nutritional status, dialysis adequacy, and residual renal function on the response to hepatitis B vaccination in peritoneal dialysis patients. Adv Perit Dial 1996; 12:315.
9 Schwebke J, Mujais S. Vaccination in hemodialysis patients. Int J Artif Organs 1989; 12:481.
10 Kausz AT, Gilbertson DT. Overview of vaccination in chronic kidney disease. Adv Chronic Kidney Dis 2006; 13:209.
11 Centers for Disease Control and Prevention. General recommendations on immunization. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 2011; 60 (No. RR-2): 1–61.
12 World Health Organisation (WHO). Recommendations on Pandemic (H1N1). 2009/2011.
13 Guidance on harmonisation of requirements for influenza vaccines. CPMP/BWP/214/96. (12 March 1997).
14 Labriola L, Hombrouck A, Maréchal C et al. Immunogenicity of an adjuvanted 2009 pandemic influenza A (H1N1) vaccine in haemodialysed patients. Nephrol Dial Transplant 2011; 26:1424–1428.
15 Crespo M, Collado S, Mir M et al. Efficacy of Influenza A H1N1/2009 Vaccine in Hemodialysis and Kidney Transplant Patients. Clin J Am Soc Nephrol 2011; 6:2208–2214.
16 Roman F, Vaman T, Kafeja F, Hanon E, Van Damme P. AS03(A)-Adjuvanted influenza A (H1N1) 2009 vaccine for adults up to 85 years of age. Clin Infect Dis 2010; 51:668–677.
17 Matas AJ, Gillingham KJ, Humar A et al. 2202 kidney transplant recipients with 10 years of graft function: what happens next? Am J Transplant 2008; 8:2410.
18 WHO recommendations on pandemic (H1N1) 2009 vaccines. http://www.who.int/csr/disease/swineflu/notes/h1n1_vaccine_20090 713/en/index.html (Accessed 13 July 2009).
19 Salles MJ, Sens YA, Boas LS, Machado CM. Influenza virus vaccination in kidney transplant recipients: serum antibody response to different immunosuppressive drugs. Clin Transplant 2010; 24:E17–E23.
20 Agarwal N, Ollington K, Kaneshiro M, Frenck R, Melmed GY. Are immunosuppressive medications associated with decreased responses to routine immunizations? A systematic review. Vaccine 2012; 30(8):1413–1424.
21 White-Williams C, Brown R, Kirklin J et al. Improving clinical practice:should we give influenza vaccinations to heart transplant patients? J Heart Lung Transplant 2006; 25:320–323.
22 Lawal A, Basler C, Branch A, Gutierrez J, Schwartz M, Schiano TD. Influenza vaccination in orthotopic liver transplant recipients: Absence of post administration ALT elevation. Am J Transplant 2004; 4:1805–1809.
23 Zhu FC, Wang H, Fang HH et al. A novel influenza A (H1N1) vaccine in various age groups. N Engl J Med 2009; 361:2414–2423.