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ORIGINAL ARTICLE

Antibody persistence 1 year after pandemic H1N1 2009 influenza vaccination and immunogenicity of subsequent seasonal influenza vaccine among adult organ transplant patients

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Summary

We investigated the antibody persistence in solid organ transplant (SOT) recipients 1 year after immunization with two doses of monovalent AS03-adjuvanted influenza A(H1N1)pdm09 vaccine. We also assessed the boosting effect of the seasonal trivalent inactivated vaccine 2010 (TIV/10) that contained the influenza A (H1N1)pdm09 strain. A total of 49 SOT recipients and 11 healthy controls were included. After a blood sample was obtained to assess the persistent immunity, one dose of TIV/10 was administered and another blood sample was collected 1 month after vaccination. A(H1N1)pdm09 antibodies were measured using a haemagglutination inhibition assay. The percentage of SOT recipients with protective titres decreased between 1 month and 10–14 months after the monovalent influenza A(H1N1)pdm09 vaccination, from 79% ($n = 38$) to 47% ($n = 23$) ($P = 0.02$). The corresponding numbers for the control group were 100% and 63%, respectively ($P = 0.008$). After the TIV/10 boosting dose, the number of SOT recipients with protective titres increased from 47% ($n = 23$) to 71% ($n = 35$) ($P = 0.2$). All the controls reached a protective titre level. The median titre rise was significantly higher among controls when compared to SOT recipients ($P = 0.0036$). No rejection or adverse events were seen. The results show an obvious need for vaccine boosting doses in the SOT patients. (ClinicalTrials.gov number: NCT01256931).

Introduction

Solid organ transplant (SOT) recipients have a higher risk of influenza infection complications when compared to healthy individuals. This problem was evidenced in 2009 by the severity of pandemic influenza A/H1N1 (A(H1N1) pdm09) infection among SOT recipients [1–3]. Kumar *et al.* [3] reported severe complications, that is, pneumonia, acute renal failure and a mortality rate of up to 7%. Smud *et al.* [4] had a similar mortality rate. That study reported a mortality rate among SOT recipients of 7.8% compared with 5.5% in the general population during the early phase of the pandemic influenza when no vaccine was available. A study which assessed natural influenza A (H1N1)pdm09 infection found that about 20% of the SOT recipients in the study lacked both humoral and cellular immunity 1 year after infection and were thus at risk of natural re-infection during the following season [5]. All of these findings demonstrate that effective immunization of immunocompromised hosts is of utmost importance.

During 2009, influenza pandemic vaccination against A (H1N1)pdm09 was recommended for SOT recipients. In Sweden, two doses of an inactivated, AS03-adjuvanted, monovalent vaccine (Pandemrix®; GlaxoSmithKline, Brentford, UK) were used [6]. The immunogenicity of this vaccine is high in a healthy population (18–64 years) with a

seroprotection rate 3 weeks after one dose of 98% [7]. In a study performed at the Transplant Institute, Sahlgrenska University Hospital, we found that this vaccine elicited a protective antibody response in 80% of SOT recipients as measured 1 month after the second vaccine dose as compared to 100% in our healthy controls [8]. Several studies of antibody responses after A(H1N1)pdm09 vaccination have reported a broad range of seroprotection in SOT patients [9–15].

As the strain influenza A(H1N1)pdm09 will likely continue to circulate for several years into the future, studies are needed to evaluate long-term immune response after vaccination.

The aim of this study was to explore both the persistence of H1N1 antibodies 1 year after immunization with an A (H1N1)pdm09 vaccine and the immune response to the H1N1 component in boosting dose of seasonal trivalent inactivated vaccine 2010 (TIV/10) in SOT recipients.

Materials and methods

Subjects and study design

Solid organ transplant recipients and healthy controls previously immunized with two doses of the monovalent AS03-adjuvanted influenza A(H1N1)pdm09 vaccine, Pandemrix® were included in this study. The SOT recipients were recruited from the outpatient clinic at the Transplant Institute, Sahlgrenska University Hospital, and the healthy controls were members of the staff. All had participated in an earlier study of antibody response to two doses of this vaccine [8]. A letter of invitation was sent to these former subjects asking them to participate in the present follow-up study, and a total of 49 SOT recipients and 11 healthy controls were included.

A blood sample was taken at visit one, 10-14 months after the last immunization with A(H1N1)pdm09 vaccine. The persistent titres of A(H1N1)pdm09 antibodies were compared with antibody titres taken 1 month after the second A(H1N1)pdm09 vaccine dose to assess the loss of protective immunity. During the same visit, a single dose of TIV/10, Fluarix® (GlaxoSmithKline) was administrated. One month later, blood samples were drawn and analysed for antibody titres to examine the boosting effect of TIV/10 on the A(H1N1)pdm09 component.

At the time of the TIV/10 vaccination, subjects received a questionnaire for the reporting of any side effects. The questionnaire was to be returned 2–3 months later. All the SOT recipients' charts were reviewed with respect to immunosuppression, graft function, performed biopsies, antirejection treatments and side effects 1 year following vaccination. In a majority of these SOT recipients, renal function was measured by the CrEDTA clearance or iohexol clearance – routinely performed annually according to the Transplant Institute's protocol. To determine whether influenza infection occurred between October 2009 and the end of 2011, all the SOT recipients' charts were reviewed concerning any influenza-related symptoms during this period. In addition, nasopharyngeal test for influenza A virus by real-time reverse transcription polymerase chain reaction (rRT-PCR) was reviewed.

Vaccine

At the study start in 2010, all the participants received the trivalent inactivated influenza vaccine (TIV/10) Fluarix® (GlaxoSmithKline) containing the A/California/7/2009 A (H1N1)pdm alike strain (NYMC X-181): 15 μg, A/Perth/16/2009 (H3N2) alike strain (NYMC X–187, derived from A/Victoria/210/2009):15 μg and B/Brisbane/60/2008: 15 μg. The vaccine was administered intramuscularly into the deltoid muscle.

In the 2009 study [8], the subjects were immunized with a monovalent, AS03-adjuvanted influenza A(H1N1)pdm09 vaccine, Pandemrix® (GlaxoSmithKline). The adjuvant AS03 was composed of squalene (10.69 mg), DL-α-tocopherol (11.86 mg) and polysorbate 80 (4.86 mg). A subset of the subjects (13 SOT recipients and 2 controls) was vaccinated during the 2009 season with TIV/09 Fluarix® vaccine (GlaxoSmithKline) contained A/Brisbane/59/2007 (H1N1) alike strain (IVR-148): 15 μg, A/Brisbane/10/2007 (H3N2) alike strain A/Uruguay/716/2007 (NYMC X-175-C): 15 μg, and B/Brisbane/60/2008 alike strain: 15 μg.

Haemagglutination inhibition assay

Pre- and postvaccination samples were analysed simultaneously by haemagglutination inhibition (HI) assay using a method described in Felldin *et al.* [8]. Briefly, the HI assay was performed with 0.5% hen erythrocytes and four haemagglutination (HA) units of virus (A/California/7/2009 NYMC X-179A H1N1). Sera were tested in serial twofold dilution steps at an initial dilution of 1:10–1:640. The efficacy of TIV/10 on boosting A(H1N1)pdm09 antibody titres was assessed using the following three indices: percentages of individuals who reached protective titres (≥1:40), percentages of individuals with a ≥fourfold titre rise, and the median titres reached in subjects.

Statistical analyses

The Fisher's exact test was used to compare SOT recipients and controls with respect to protective antibody titres (≥1:40), side effects, gender and age (< or >60 years). For the SOT recipient cohort, the correlation between protective titre versus GFR (< or > 30 ml/min) and KDOQI chronic kidney disease stages (1–5) and type of

transplant was analysed by the same method. Wilcoxon two sample test was used to compare the median titre value after the TIV/10 boosting dose in SOT recipients and controls. The McNemar's test was used to compare the proportion of responders before and after the booster vaccine dose. Finally, Pearson's correlation coefficient was used to measured correlations between titres and the different immunosuppressants. The analyses were performed using SAS software, version 9.1 (SAS Institue Inc., Cary, NC, USA).

Ethics

The Regional Ethical Review Board in Gothenburg approved the study. All the study participants gave their written informed consent prior to participation.

Results

The demographics of the 49 SOT recipients and 11 controls are shown in Table 1. The majority of the SOT recipients were renal transplanted. Except for two, all were more than 1 year post-transplantation. One liver recipient was retransplanted 6 months before immunization with TIV/10. This recipient had lost the graft because of chronic rejection that started before the A(H1N1)pdm09 vaccination. Another liver transplant recipient who was participating in the study had declining liver transplant function because of biliary complication at the time of enrolment and was retransplanted during the month between TIV/10 administration and the follow-up serum sample. The healthy controls were the members of the Transplant Institute's staff.

Table 1. Demographics of the study population.

Characteristics	Transplant recipients (n = 49)	Healthy controls (n = 11)
Age years, median (range)	59 (32–80)	42 (24–57)
Gender F/M	25/24	7/4
Organ transplanted		
Kidney	27	
Liver	13	
Heart	4	
Lung	0	
Kidney–heart/Kidney–liver	1/3	
Lung/liver	1	
Years since last transplantation		
Median (range)	7 (0–23)	
Renal function Median (range)*	47 (9–110)	
mGFR/eGFR*		
>60 (CKD stage 1 + 2)	6/7	
30–59 (CKD stage 3)	23/3	
15–30 (CKD stage 4)	5/0	
<15 (CKD stage 5)	5/0	
Immunosuppressive therapy (%)		
Cyclosporine	13 (26)	
Tacrolimus	30 (61)	
Mycophenolate acid	28 (57)	
Azathioprine	2 (4)	
PSI	3 (6)	
Corticosteroids	35 (71)	
Belatacept	1 (2)	

*Renal function grouped according to KDOQI guidelines into chronic kidney disease (CKD) stages 1–5. Measured GFR (mGFR) with CrEDTA or Iohexol technique ml/min/1.73 m^2 in 39 patients. In 10 patients, only serum creatinine was available why we used the MDRD formula to estimate GFR (eGFR).

Enrolment and follow-up of study participants are shown in Fig. 1.

There was a great diversity with respect to basal immunosuppression among SOT recipients (Table 1). The kidney recipients were most often on triple therapy (15 of 27, 55.5%) consisting mainly of calcineurin inhibitors (CNI), mycophenolate acid (MPA) and steroids. The liver recipients were treated with three drugs (n = 4), two drugs (n = 6) or single therapy (n = 3). Three of the four heart transplant recipients were treated with CNI in combination with one other drug (azathioprine, MPA and proliferation signal inhibitors (PSI) respectively). The fourth was on triple therapy. Immunosuppression remained unchanged between vaccination with the A(H1N1)pdm09 vaccine and the TIV/10 booster except for the liver re-transplant patient, two liver and one renal recipients who were weaned off steroids, and another renal recipient close to dialysis where MPA was reduced. No acute rejection was seen during the study period.

Antibody persistence 1 year after influenza A(H1N1) pdm09 vaccination

In 2009, all the SOT recipients and controls were vaccinated with two doses of AS03-adjuvanted influenza A (H1N1)pdm09 vaccine. Only one, a SOT recipient had a seroprotective titre (1:40) before the A(H1N1)pdm09 vaccination. There was a significant loss of protective titres 10–14 months after A(H1N1)pdm09 vaccination in both SOT recipients and controls. One month after A(H1N1)pdm09 vaccination, a total of 38 (79%) SOT recipients had protective HI titres compared with 23 (47%) after 10–14 months (P = 0.02). The corresponding numbers for the control group were 11 (100%) and 7 (63%), respectively (P = 0.008) (Fig. 2).

Efficacy of TIV/10 on boosting A(H1N1)pdm09 antibody titres

After the boosting immunization with TIV/10, the proportion of SOT recipient with protective titres (≥1:40) against A(H1N1)pdm09 increased from 23 (47%) to 35 (71%), a trend towards better protection but not significant

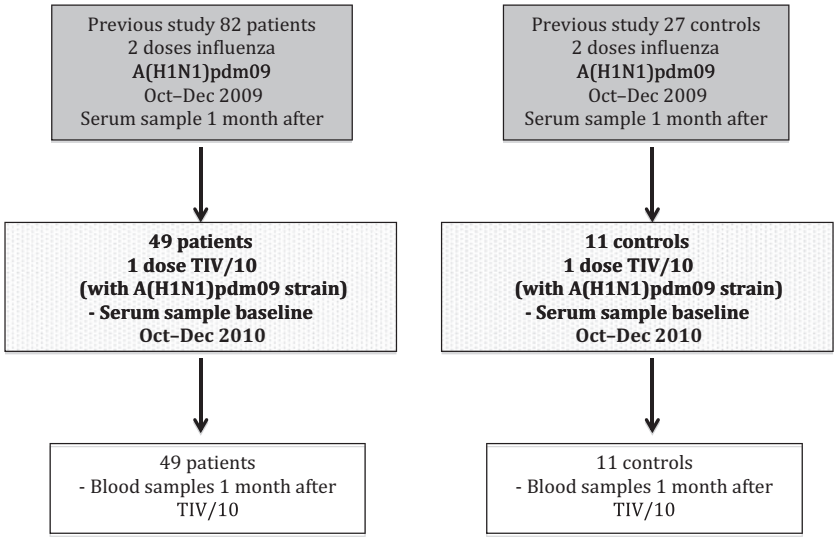

Figure 1 A schematic view of former and this study.

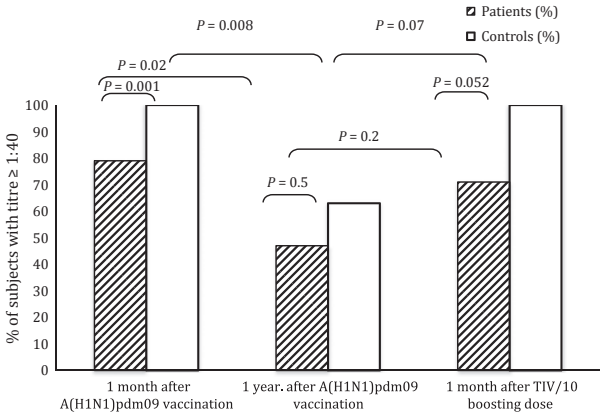

Figure 2 Percentages of organ transplant patients vs. healthy controls with protective serum haemagglutination inhibition antibody titres (≥ 1:40). Left bars: after two doses of influenza A(H1N1)pdm09 vaccination. Middle bars: 10–14 months later. Right bars: after the seasonal influenza vaccination booster dose (TIV/10).

($P = 0.2$). All the control individuals reached protective antibody titre levels (Fig. 2).

The titre rise was at least fourfold in 22 (41%) of the SOT recipients; an additional two recipients already had a maximal titre (≥ 1:640) before TIV/10 (data not shown). The corresponding figures for the 11 controls were eight with at least a fourfold rise and three who already had a maximum titre.

The distribution of the magnitude of the A(H1N1)pdm09 antibody titre rise after boosting with TIV/10 is shown in Fig. 3. The median titre increased from 1:20 to 1:80 in the SOT recipients and from 1:80 to 1:640 among the controls. The titre rise was significantly lower among the SOT recipients when compared to the controls ($P = 0.0036$).

Among the SOT recipients, we also found a significant relationship between those with protective titre after A(H1N1)pdm09 vaccination and those who once again reached protective titres after a boosting dose of TIV/10 ($P = 0.0002$).

During the 2009 season, six SOT recipients and one control received TIV/09 together with the second dose of A(H1N1)pdm09 vaccine and seven SOT recipients and one control after the termination of the A(H1N1)pdm09 study. We did not analyse cross-reactive antibodies. Excluding these individuals from the analysis yielded equivalent results; 55% of patients had remaining immunity 10–14 months after A(H1N1)pdm09 vaccination and 72% after TIV/10.

Protection against influenza infection

No episode of influenza and no positive rRT-PCR results for influenza from nasopharyngeal samples were recorded among the SOT recipients from October 2009 until the end of 2011.

Nonresponders to the A(H1N1)pdm09 component of TIV/10

Fourteen subjects were nonresponders (HI titre < 1:40) to the H1N1 component of TIV/10; all were in the SOT recipient group. Of the 26 SOT recipients who did not have protective titres before TIV/10, 13 developed protective ti-

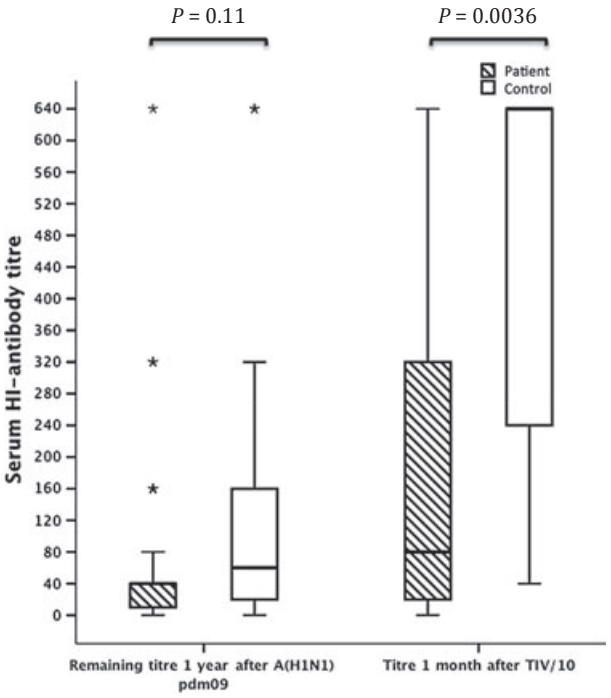

Figure 3 The box plot showing the distribution of the magnitude of H1N1 antibody titre in organ transplant patients versus healthy controls 10–14 months after two doses of influenza A(H1N1)pdm09 vaccination (left bars) and 1 month after the seasonal influenza vaccination booster dose (TIV/10). The box plot represents the 25–75th percentile, the dark line is the median, and the extended bars represent the 10–90th percentile. An asterisk represents suspected outliers.

Table 2. Frequency of side effects after TIV/10 reported in questionnaires by transplant patients and healthy controls.

Reported symptoms	Patients (n = 37)	Controls (n = 11)
Local symptoms n (%)	8 (21)	4 (36)
Myalgia n (%)	7 (19)	3 (27)
Fever n (%)	2 (5.4)	0
Cough n (%)	4 (11)	0
Headache n (%)	1 (2.7)	0

tres and one lost seroprotection. The latter was the patient who received a new liver graft between TIV/10 vaccination and follow-up. The other SOT recipient who received the boosting dose less than 1 year after liver transplantation (6 months) responded well (titre from 1:160 to 1: 640).

These 14 nonresponders did not differ regarding age (>60 years), gender, transplanted organ or time after transplantation when compared to responders. However, the nonresponders had a lower renal function (GFR < 30 ml/min/1.73 m^2) (P = 0.003) and were more often treated with three immunosuppressive drugs (P = 0.009) when compared to the responders. Of the nonresponders, 13 (93%) were treated with MPA compared with 17 (48.6%) of the responders (P = 0.0006). As a result of the small number of patients, a multivariate analysis was not possible.

Side effects

Side effects were assessed using the questionnaires distributed to all participants. 37 of the 49 (75%) SOT recipients and all of the controls returned the questionnaires. The frequency of side effects is shown in Table 2. All side effects reported by the participants were mild and did not require medical attention. The study was, however, not designed to investigate the safety of the vaccine.

Rejection

No acute rejection episode occurred during 1-year follow-up after TIV/10 vaccination. During this period, no biopsies for protocol or any other clinical indications were performed. Immunosuppression remained unchanged except for two SOT recipients: one was retransplanted and the other had chronic rejection.

Discussion

In the present study, we found a significant loss of protective HI antibody titres against influenza A(H1N1)pdm09 1 year after vaccination with two doses of monovalent, AS03-adjuvanted influenza A(H1N1)pdm09 vaccine (Pandemrix®). This was the case among both SOT recipients and healthy controls.

Recently, two studies have been published on the loss of the antibody protection 1 year after using A(H1N1)pdm09 vaccine in SOT recipients [16,17]. Cordero *et al.* [16] reported that protection declined from 80 to 30% over 1 year; seemingly a more profound drop than in our study. In that study, only one dose of MF69-adjuvanted monovalent vaccine was administered. To some extent, this could account for the lower remaining immunity in their cohort of recipients. Siegrist *et al.* [17] used two doses of AS03-adjuvanted influenza vaccine – as in our study. They found that 67% of the kidney transplant recipients had protective titres 1 month after immunization. In contrast to our study, they did not detect a loss of protection as 65% continued to have HI titres of ≥ 1:40 1 year after vaccination. There is no obvious explanation for this difference when compared to our study as they studied renal recipients with comparable age, immunosuppression, time after transplantation and renal function.

The boosting with TIV/10 enhanced the immune response against A(H1N1)pdm09 in our SOT recipients to some extent although not significantly. In particular, the frequency of responders increased from 49 to 71% as com-

pared with 100% among the healthy controls. Other studies have also shown a booster effect with TIV/10 among the organ transplant populations. In these studies, the seroprotection rate after TIV/10 vaccination varied between 53% and around 80% [16–18]. The lower frequency (53%) of responders reported in the study performed by Mulley *et al.* [18] may reflect the low baseline titres after a single dose of nonadjuvanted A(H1N1)pdm09 vaccine.

One-third of our SOT recipients did not reach a protective antibody titre level after TIV/10 (i.e. nonresponders). These study participants did not differ regarding age, gender or transplanted organ when compared to responders. However, the nonresponders had a significantly lower renal function and were significantly more likely to be on triple immunosuppressive therapy when compared to the responders. Additionally, we found a significant correlation between MPA treatment and nonresponders. This is in line with a study on TIV effect in renal transplant recipients that was performed before the pandemic influenza [19]. In that study, MPA treatment was the strongest predictive factor in a multivariate analysis of those not reaching protective antibody levels. Recently, Mulley *et al.* [18] found that MPA treatment reduced the likelihood of achieving seroprotection after A(H1N1)pdm09 vaccination in a dose-dependent manner. As in our study, Mulley *et al.* also found a significant correlation between low responsiveness and GFR < 30 ml/min.

We did not study cross-reactive antibodies against A(H1N1)pdm09. However, the protection rate remained the same when the 13 SOT recipients and two controls that received TIV/09 were excluded from the analysis. The proportion of patients receiving TIV/09 was low when compared to other studies [16,17]. Hancock *et al.* [20] reported that seasonal influenza vaccines induced little or no cross-reactive antibody response to A(H1N1)pdm09. This could be explained by the large degree of genetic divergence of the pandemic H1N1 viruses of swine origin as compared to recent H1N1 viruses [21]. Thus, it appears that the influence of the seasonal 2009 TIV vaccine on antibody response against A(H1N1)pdm09 was low.

In a transplant population, it is important that any treatment, including vaccines, does not elicit a rejection. No acute rejection episodes occurred in our patient cohort. The immunization with seasonal influenza vaccine has not been reported to cause rejection [19,22] or HLA-antibody development [23]. The AS03-adjuvanted influenza A(H1N1)pdm09 vaccine seems, however, to be more immunogenic, and all our SOT recipients had previously been vaccinated with this vaccine and then boosted with A(H1N1)pdm09 alike component. After the A(H1N1)pdm09 vaccination, Katerinis *et al.* [24] reported the development of donor-specific HLA-antibodies, but no correspondent clinical rejection in renal-transplanted patients. Schaffer *et al.* [25] retrospectively compared heart transplant recipients immunized with AS03-adjuvanted influenza A(H1N1)pdm09 with nonvaccinated recipients; all of whom were undergoing regular surveillance endomyocardial biopsies. There were six of 15 vaccinated and one of 45 nonvaccinated patients who had a rejection $\geq$grade 2. All these cellular rejections were treatable, and de novo donor-specific antibodies were not discovered. In a different study, it was observed that the A(H1N1)pdm09 infection by itself triggered rejection in one pancreas transplant recipient [26]. As reported previously [8], one of our renal transplant recipients was diagnosed with chronic rejection after the AS03-adjuvanted influenza A(H1N1)pdm09 vaccination, but no acute rejection episodes were evident after either the former or current vaccination. The long-term consequences of vaccination are, of course, unknown.

Diverging results in studies on SOT recipients highlights the difficulties encountered when comparing results. The relatively low number of participants as well as different vaccines and dosage regimens likely influenced outcomes. Immunosuppressive protocols may have varied between individuals and transplant centres. Also, the recipient cohorts may have differed with respect to renal impairment and other comorbidity. We have made comparisons between SOT recipients and a group of healthy controls. However, because of the small number of controls in our study, the results have to be interpreted with caution.

Finally, one should keep in mind that the chosen value of the titre 1:40 as a threshold for immunity is putative and should be used with care, especially in an immunocompromised host.

In summary, 1 year after immunization with two doses of the AS03-adjuvanted influenza A(H1N1)pdm09 vaccine approximately one-third of both the SOT recipients and controls had lost their protective antibody level – although the vaccine had elicited an immediate strong immune response the year before. A boosting dose of TIV/10 resulted in a protective titre increase from 47 to 71% among SOT recipients. The nonresponsiveness correlated with a lower renal function, triple immunosuppression and MPA treatment. No rejection was seen. If the strain A(H1N1)pdm09 continues to circulate for several years into the future, there is an on obvious need for vaccine boosting doses in the SOT recipient population.

Authorship

MF, VF and BS: designed and performed the study. MF: collected the data. BS: performed the virological analyses. MF, BA, MS, BS and VF: analysed the data and wrote the paper.

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