Mycophenolate and lower graft function reduce the seroresponse of kidney transplant recipients to pandemic H1N1 vaccination

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In late 2009 transplant organizations recommended that kidney recipients be vaccinated for pandemic H1N1 influenza (pH1N1); however, the vaccine efficacy was unknown. We had offered a monovalent non-adjuvanted pH1N1 vaccine to transplant recipients. Here we compared the pre- and post-vaccination seroresponses of 151 transplant recipients to that of 71 hemodialysis patients and 30 healthy controls. Baseline seroprotection was similar between groups but was significantly different at 1 month (44, 56, and 87%, respectively). Seroconversion was significantly less common for transplant recipients (32%) than dialysis patients (45%) and healthy controls (77%). After adjusting for age and gender, dialysis patients were significantly more likely (2.7-fold) to achieve new seroprotection than transplant recipients. The likelihood of seroprotection in transplant recipients was significantly reduced by mycophenolate use (adjusted odds ratio 0.24), in a dose-dependent manner, and by reduced eGFR (adjusted odds ratio 0.16 for worst to best). Seroprotection and geometric mean antibody titers increased substantially in 49 transplant recipients who subsequently received the 2010 seasonal influenza vaccine. Thus, patients requiring renal replacement therapy had reduced seroresponses to vaccination with the monovalent vaccine compared with healthy controls. Transplant recipient responses were further reduced if they were receiving mycophenolate or had significantly lower graft function.

Kidney International (2012) 82, 212–219; doi:10.1038/ki.2012.106; published online 11 April 2012

KEYWORDS: efficacy; eGFR; hemodialysis; serology; swine flu; vaccine

An influenza pandemic, generated by a novel strain of influenza A (pH1N1), was declared by the World Health Organization in June 2009.1 Low levels (<20%) of preexisting seroprotective antibody titers in people from most countries, including Australia, against pH1N1 were due to antigenic differences between the pre-pandemic seasonal H1N1 strains previously circulating in humans and the pH1N1 virus, which emerged from pigs.2 As a result of this antigenic difference, seasonal influenza trivalent vaccines produced before the 2009 pandemic did not result in significant rates of seroprotection against pH1N1.3–5 To address this, monovalent pH1N1 vaccines were rapidly developed and were shown to be highly effective in healthy volunteers.6–8 In September 2009, international transplant societies recommended that solid organ transplant recipients receive the monovalent vaccine along with their family members and close contacts.9 No data on the efficacy of the monovalent vaccines in kidney transplant recipients were available at that time.

Kidney transplant recipients in the era of modern immunosuppressive agents have typically demonstrated a reduced capacity to generate seroprotective antibody titers against H1N1 after seasonal trivalent influenza vaccination compared with healthy controls.10–14 Mycophenolate appears to be particularly effective at reducing rates of seroprotection.11,13–15 Although seroresponses are reduced in kidney transplant recipients, seroprotection is achieved in a significant percentage, and this may be increased in centers that encourage annual vaccination.13 As such, the seasonal trivalent influenza vaccination is recommended for kidney transplant recipients from 3 to 6 months post transplant.16
We accepted the advice to immunize our kidney transplant recipients against pH1N1 in late 2009. Although three recent studies have reported impaired seroresponses to adjuvanted pH1N1 vaccines in kidney transplant recipients, we now present the first data regarding seroresponses to a monovalent non-adjuvanted pH1N1 vaccine in a larger cohort of kidney transplant recipients. Their responses are compared with a hemodialysis patient population and healthy controls with a focus on those kidney transplant recipients at greatest risk of pH1N1 infection on the basis of having no baseline seroprotection. The overall low baseline seroprotection rates in the community provided the opportunity to test the hypothesis that there is a stepwise hierarchy of seroresponses from healthy controls with best responses, through hemodialysis patients with intermediate responses, to kidney transplant recipients with the poorest responses, and furthermore that transplant recipients’ responses are modulated by renal function and mycophenolate use. We also offer support to the recommendation of seasonal influenza vaccination in transplant recipients by demonstrating a boosting effect in those who received the 2010 influenza vaccine after receiving the 2009 monovalent pH1N1 vaccine.

RESULTS
Participants
In all, 526 kidney transplant recipients were approached for inclusion in the study (Figure 1). Of these, 287 patients were excluded from further participation: 75/526 (14.3%) declined to participate, 10/526 (1.9%) were deemed unsuitable because of active illness or transplantation within 21 days, 105/526 (20.0%) did not respond to phone or postal invitations for vaccination, and 97/526 (18.4%) received vaccination from their family doctor. Of the 526 subjects, 239 (45%) received the monovalent pH1N1 vaccine at our center and had pre-vaccination pH1N1 serology performed. Pre-vaccination and 1 month post-vaccination serology were available in 151/239 (63.2% of the cohort vaccinated at our center).

In all, 82 hemodialysis patients attending two satellite hemodialysis facilities had pre-vaccination serology and received the monovalent pH1N1 vaccine (Figure 1); 71 had 1 month post-vaccination serology. Pre- and post-vaccination sera were available from 30 healthy control participants.

Basic demographics of the kidney transplant and hemodialysis patients are presented in Table 1. The kidney transplant recipients were younger, with a greater proportion of female recipients compared with the hemodialysis patients. Glomerulonephritis was the most common cause of end-stage kidney failure in the transplant recipients, whereas diabetes mellitus was more common in the hemodialysis patients. The healthy controls were significantly younger on average and had a higher proportion of female participants than both the renal replacement groups. They had no significant medical conditions.

Baseline seroprotection
Pre-vaccination antibody titers were available in 239 kidney transplant recipients, 82 hemodialysis patients, and 30 healthy controls. The baseline level of seroprotection was not different between the three groups, with 35 (14.6%) kidney transplant recipients, 13 (15.9%) hemodialysis patients, and 3 (10%) healthy controls with titers \( \geq 40 \) (\( P = 0.74 \)) (Table 2).

Seroresponses
Pre- and post-vaccination serology was available in 151 kidney transplant recipients. In all, 20/151 (13%) had seroprotection at baseline, and this increased to 66/151
(44%) at 1 month post vaccination (Table 2). Of the 71 hemodialysis patients who completed pre- and post-vaccination serology, there was a similar level of baseline seroprotection (16%), but a greater level of seroprotection at 1 month (56%) (Table 2). The healthy controls had the largest response, with baseline and 1 month seroprotective rates of 10 and 87%, respectively (Table 2). Seroprotective titers at 1 month were significantly different between groups (P<0.01) (Table 2). Similarly, geometric mean antibody titers were not different at baseline (kidney transplant = 9, hemodialysis = 11, and healthy controls = 8), but were significantly different at 1 month (kidney transplant = 26.8, hemodialysis patients = 53.1, and healthy controls = 121.3; P<0.001) (Table 2). Finally, seroconversion (titer ≥ 40 and a ≥ 4-fold increase in titer) was significantly less common in transplant recipients compared with hemodialysis patients and healthy controls (31.8, 45.1, and 77%, respectively; P<0.001) (Table 2).

In those without seroprotection at baseline, the same trend was observed with transplant recipients having significantly lower seroprotection and seroconversion rates with lower geometric mean antibody titers at 1 month compared with hemodialysis patients and healthy controls (Table 2).

After adjustment for age and gender, hemodialysis patients were more than two times as likely (adjusted odds ratio 2.68, 95% confidence interval 1.31–5.52, P = 0.007) and healthy controls six times as likely to achieve seroprotection (aOR 5.84, CI 1.79–19.06, P = 0.003) than transplant recipients (Table 3). Advancing age was associated with a reduced likelihood of achieving new seroprotection (Table 3). Cause of end-stage renal failure was not associated with achieving seroprotection.

### Transplant recipients

The demographic details, comorbidities, and immunosuppressive regimens are summarized in Table 4. The median time since transplant was 3.7 years (range 0.1–27.9 years). Most (137 (90.7%)) were primary grafts, whereas 11 (9.3%) and 3 (2%) were second and third grafts, respectively. The majority of recipients (90%) were receiving a calcineurin inhibitor (65% tacrolimus and 35% cyclosporine). Mycophenolate use was by 82%, whereas 64% were taking prednisolone. Median transplant function was 49 ml/min per 1.73 m² with 9, 52, and 39% with an estimated glomerular filtration (eGFR) of 0–30, 31–60, and ≥60 ml/min per 1.73 m², respectively. In total, 68 (45%) patients had received the 2009 seasonal trivalent influenza vaccine.

Univariate and multivariate analyses of the transplant cohort without baseline seroprotection (n = 131) was undertaken to determine which factors influenced the likelihood of new seroprotection (Table 5). Mycophenolate use was independently associated with a 76% reduced likelihood of achieving seroprotection (aOR 0.24, CI 0.07–0.79, P = 0.02) (Table 5 and Figure 2a). A dose effect of mycophenolate on response rates was apparent after adjusting for recipient age and gender, transplant duration, calcineurin inhibitor use, prednisolone use, and rituximab use. Patients on ≥2 g per

### Table 1 | Characteristics of renal replacement study groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Kidney transplant</th>
<th>Hemodialysis</th>
<th>Healthy control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>84 (55.6)</td>
<td>47 (66.2)</td>
<td>10 (33.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>Age (years)</td>
<td>51.2 ± 12.6</td>
<td>62.6 ± 12.8</td>
<td>32.6 ± 13.1</td>
<td>&lt;0.001</td>
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<tr>
<td><strong>Primary kidney disease</strong></td>
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</tr>
<tr>
<td>Glomerulonephritis</td>
<td>84 (56)</td>
<td>17 (24)</td>
<td></td>
<td>&lt;0.001</td>
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<tr>
<td>Diabetes mellitus</td>
<td>21 (14)</td>
<td>31 (44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension/ischemic</td>
<td>8 (5)</td>
<td>3 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADPCKD</td>
<td>20 (13)</td>
<td>4 (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reflux nephropathy</td>
<td>18 (12)</td>
<td>4 (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (3)</td>
<td>12 (17)</td>
<td></td>
<td></td>
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<tr>
<td><strong>Seroprotection, n (%)</strong></td>
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<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>20 (13.2)</td>
<td>11 (15.5)</td>
<td>3 (10)</td>
<td>0.75</td>
</tr>
<tr>
<td>1 month</td>
<td>66 (43.7)</td>
<td>40 (56.3)</td>
<td>26 (86.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Seroprotection, n (%)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Geometric mean</td>
<td>9.3</td>
<td>11.1</td>
<td>7.8</td>
<td>0.21</td>
</tr>
<tr>
<td>1 month</td>
<td>26.8</td>
<td>53.1</td>
<td>121.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Seroconversion, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>48 (31.8)</td>
<td>32 (45.1)</td>
<td>23 (77)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1 month</td>
<td>47 (35.9)</td>
<td>29 (48.3)</td>
<td>23 (85)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### Table 2 | Anti-pH1N1 serology at baseline and 1 month after vaccination

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Kidney transplant</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>1.67</td>
<td>0.90–3.1</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>10.28</td>
<td>3.35–31.5</td>
</tr>
<tr>
<td>Age (per 10 year increase)</td>
<td>0.69</td>
<td>0.57–0.84</td>
</tr>
<tr>
<td>Female gender</td>
<td>1.21</td>
<td>0.71–2.07</td>
</tr>
</tbody>
</table>

### Table 3 | Predictors of achieving seroprotection

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
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</tbody>
</table>

**Abbreviations:** ADPCKD, autosomal dominant polycystic kidney disease; HDP, hemodialysis patients; KTR, kidney transplant recipients; pH1N1, pandemic H1N1 influenza.
day were significantly less likely to achieve seroprotection than those on <2 g per day and those taking no mycophenolate (20, 34, and 50%, respectively, *P* = 0.026 for trend) (Figure 3).

The use of other immunosuppressive agents, including prednisolone and calcineurin inhibitors, did not independently predict failure to achieve seroprotection; however, only 1 of 10 patients who had received rituximab achieved seroprotection, suggesting reduced *de novo* antibody responses with its use. The patient who did achieve seroprotection received rituximab 3.5 years prior and had normal B-cell counts at vaccination.

There was an incremental decline in the likelihood of achieving seroprotection with declining transplant function. Patients with an eGFR <30 ml/min per 1.73 m² were significantly less likely to achieve seroprotection compared with those with an eGFR ≥60 ml/min per 1.73 m² (OR 0.16, 95% CI 0.03–0.88, *P* = 0.04; Table 5 and Figure 2b). Advancing age per 10-year increment (aOR 0.70, CI 0.49–0.996, *P* = 0.047) and shorter transplant duration (<6 vs. ≥6 months) (aOR 0.10, CI 0.01–0.86, *P* = 0.04) were also associated with a reduced likelihood of achieving seroprotection (Table 5).

**Boosting effect of the 2010 trivalent influenza vaccine**

Of the 151 transplant recipients, 49 (32.4%) received the 2010 seasonal trivalent influenza vaccine, which included antigens specific to the 2009 pH1N1 virus and had their antibody titers against pH1N1 repeated. This subset of patients was similar to the group as a whole. Their mean age was 55.3 ± 10.7 years, 59% were men, and 61% had glomerulonephritis as the cause of their end-stage kidney failure. Most (90%) were primary grafts, and the median transplant duration was 4.9 years (range 0.13–22 years). Most were on calcineurin inhibitors (88%) and mycophenolate (80%), whereas 71% were taking prednisolone. The mean eGFR of this group was 49.3 ml/min per 1.73 m² (range 23–92.3 ml/min per 1.73 m²). The median time between the two vaccines was 7.4 months (range 3.3–10.5 months).

Seroprotective antibody titers rose in a stepwise manner from 16.3% before the 2009 pH1N1 monovalent vaccine to 34.7% 1 month post vaccination, increasing further to 53.1% after the 2010 seasonal influenza trivalent vaccine. In addition, the geometric mean antibody titers increased from 9.3 before vaccination to 20.3 after the 2009 monovalent vaccine and further to 31.0 after the 2010 trivalent vaccine.

**Safety.** No significant adverse events were reported by those undergoing vaccination; in particular, no patient required admission or had a hypersensitivity response related to the vaccine.

**DISCUSSION**

The major findings of this study were that patients requiring renal replacement therapy either in the form of hemodialysis...
or a kidney transplant had reduced seroresponses to vaccination with the monovalent vaccine compared with healthy controls. Transplant recipient responses were further reduced if the patient was receiving mycophenolate as part of their immunosuppressive regimen or had significantly lower graft function (eGFR < 30 ml/min per 1.73 m²).

Responses to vaccination such as influenza20,21 and hepatitis B (reviewed in Edey et al.22) have previously been reported to be diminished in dialysis patients, whereas studies in kidney transplant recipients have demonstrated impaired responses to influenza vaccination in the era of tacrolimus- and mycophenolate-based immunosuppressive regimens.10–14 The adjuvanted and non-adjuvanted monovalent vaccines developed in response to the 2009 H1N1 pandemic resulted in excellent seroresponses in healthy controls, with approximately 90% achieving seroprotection post vaccination.6–8,23 These rates are consistent with the healthy controls in our study.

Two recent publications describe seroresponses to different adjuvanted monovalent vaccine preparations for pH1N1 in dialysis patients. Seroconversion was almost identical (64.2 and 64.1%) in both studies after a single dose.20,21 Although not clearly stated in either study, baseline seroprotection levels appear to be < 30%, giving an increment after vaccination of approximately 30%, which was significantly less than that for healthy controls. Our observations using a non-adjuvanted preparation are in keeping with these studies, with an increment in seroprotection of 39.8% to a total of 56.3%. The combination of this study and those of Dikow and Labriola suggest that hemodialysis patients have significantly impaired responses to pH1N1 vaccination at approximately half that of healthy controls. An improved response rate may be achieved by booster dosing, however, with Dikow et al.20 demonstrating a 24% greater seroprotection rate in patients administered a second dose of the vaccine 21 days after the first.

Previous studies of trivalent seasonal influenza vaccination in kidney transplant recipients describe seroresponses against pre-pandemic seasonal H1N1 ranging from very poor to equivalent to healthy controls.10–15 Most of these studies have examined single-dose vaccination. Seroprotection post vaccination is a commonly reported efficacy outcome; however, this is dependent to a large extent on pre-vaccination seroprotection levels. For example, post-vaccination seroprotection in the study by Scharpe et al.13 was 92.7%, which exceeded that of the healthy controls (70.7%); however, baseline seroprotection was already evident in 78.2% of transplant recipients (as a result of an annual vaccination policy) compared with 25% of controls, making comparisons of efficacy difficult. Interestingly, seroconversion was similar for kidney transplant recipients and healthy controls. In this
study, the absence of significant baseline seroprotection meant that responses to vaccination could be more clearly assessed.

Most previous studies comparing vaccination outcomes in kidney transplant recipients and healthy controls have demonstrated significantly lower seroresponses and geometric mean antibody titers in transplant recipients.\textsuperscript{10–12,14,15} Previous studies had not compared seroresponses of kidney transplant recipients with those of hemodialysis patients; however, two recent studies using adjuvanted pH1N1 vaccines have now done so.\textsuperscript{18,19} The first found no statistically significant difference between groups but may have been limited by a small sample size,\textsuperscript{19} whereas the second study with larger numbers described statistically significant differences in response rates, being greatest for healthy controls, less for dialysis patients, and least for transplant recipients.\textsuperscript{18} We demonstrate the same trend using a non-adjuvanted vaccine wherein the inferior response rates in kidney transplant recipients retained significance after controlling for age and gender. This difference in responses is likely due to the use of immunosuppressive medications and in particular mycophenolate.

After controlling for other immunosuppressive medication use, age, gender, and transplant duration, treatment with mycophenolate reduced the likelihood of achieving seroprotection in the transplant recipients without baseline seroprotection by 76%. Furthermore, after adjusting for the above potential confounding factors, there was a statistically significant dose effect seen in those receiving mycophenolate. Previous studies have reported this phenomenon to varying degrees.\textsuperscript{12–15} Smith et al.,\textsuperscript{14} reported no seroconversion to influenza A in patients taking mycophenolate who were given the seasonal influenza vaccine. Salles et al.,\textsuperscript{15} demonstrated a 50% lower seroconversion rate after influenza A vaccination in transplant recipients taking mycophenolate compared with those taking azathioprine. Scharpe et al.,\textsuperscript{13} found a similar reduction in seroprotection in kidney transplant recipients on mycophenolate while also demonstrating a dose effect with reduced seroresponse rates in those receiving ≥2 g per day compared with <2 g per day. Sanchez-Fructuoso et al.,\textsuperscript{12} did not see a difference in seroprotection rates at 1 month post vaccination in patients taking mycophenolate compared with those taking azathioprine, but there was a 50% lower rate of seroprotection in those on mycophenolate at 3 months post vaccination. Similarly, in three recent studies using adjuvanted pH1N1 vaccination, the mean mycophenolate dose was higher in non-responders compared with responders in one study;\textsuperscript{17} a second study described higher trough levels in non-responders albeit not statistically significant,\textsuperscript{19} whereas the third demonstrated suppressed mean antibody titers in mycophenolate-treated patients.\textsuperscript{18} Mycophenolate inhibits both T- and B-cell proliferation by depleting guanosine nucleotides through inhibition of the enzyme inosine monophosphate dehydrogenase, which is expressed preferentially in activated lymphocytes.\textsuperscript{24} This mechanism differs from that of azathioprine and may contribute to differences in antibody responses. Taken together, this collection of data, strengthened by this study, indicates an independent and dose-dependent effect of mycophenolate on antibody responses to adjuvanted and non-adjuvanted influenza vaccination.

Reduced eGFR modified the probability of achieving seroprotection in kidney transplant recipients without baseline seroprotection. In all, 90% of healthy controls (no renal disease) without baseline seroprotection achieved seroprotection post vaccination. There was a stepwise decrease in the likelihood of achieving seroprotection in kidney transplant recipients based on their renal function, with 45% of those with satisfactory kidney graft function (eGFR >60 ml/min per 1.73 m\textsuperscript{2}), 35% with intermediate kidney function (eGFR 30–60 ml/min per 1.73 m\textsuperscript{2}), and only 15% with poor kidney function (<30 ml/min per 1.73 m\textsuperscript{2}) achieving seroprotection. Immunosuppression therefore appears to halve the odds of achieving seroprotection in transplant recipients with excellent kidney function. Worsening allograft function appears to further suppress immune responses. Given that the hemodialysis patients achieved seroprotection rates equivalent to the transplant recipients with eGFR >60 ml/min per 1.73 m\textsuperscript{2}, a possible approximate equivalence of the effects of renal failure and immunosuppression on vaccine responses is suggested, with the combination of both renal failure and immunosuppression being additive. In addition, older age and shorter transplant duration were statistically significant associates of a lower likelihood of achieving seroprotection in a multivariate analysis in the transplant recipients. Although studies including larger numbers of participants are required to confirm a suppressive effect of rituximab on seroresponses to vaccination, B-cell depletion would be anticipated to limit the generation of plasma cells specific for new antigens.

The value of booster dosing in renal transplant recipients on modern immunosuppression is unclear. Scharpe et al.,\textsuperscript{13} did not see an increase in seroprotection or seroresponses in patients given a second dose of a trivalent influenza vaccine 3 months after the initial dose; however, their patient cohort had an exceptionally high baseline level of seroprotection, which the authors suggest is likely the result of their annual vaccination policy, thereby mitigating the argument that booster dosing is ineffective. Brakemeier et al.,\textsuperscript{17} describe one additional patient attaining seroprotection against pH1N1 after a booster dose of an adjuvanted vaccine 21 days after the first and conclude that booster dosing is ineffective; however, only 19 patients were studied. Our results demonstrate a potentially important increment in the rate of seroprotection and geometric mean titers with booster dosing. Although the previous studies examined boosting with the same vaccine within the same season, this study examines the boosting effect of revaccination with a different vaccine after a longer interval period, which may have contributed to the observed differences in results. Our data suggest that annual vaccination will generate an increased rate of protection in a cohort.
of transplant recipients against antigens commonly represented in seasonal influenza vaccines, thereby supporting current recommendations. The optimal timing and frequency of booster dosing remains unclear. In addition, although boosting may increase seroprotection against known influenza strains without crossreactivity against future novel influenza variants, pandemics will not be prevented.

This study is strengthened by the inclusion of a large cohort of kidney transplant recipients with low baseline seroprotection, allowing a more detailed analysis of factors influencing seroresponses than previous vaccine studies. Inclusion of both hemodialysis patients and healthy controls is novel and has allowed demonstration of a hierarchy of seroresponses in renal failure patients. Study limitations include the following: the predominance of mycophenolate use in the kidney transplant recipients, which limited numbers in the non-mycophenolate group and restricted subanalyses, and that only 1/3 of our transplant recipient cohort took part in the study, which may have resulted in exclusion of an important subgroup of patients.

We have demonstrated that the pH1N1 monovalent vaccine, although highly effective at producing seroprotective antibody titers in healthy individuals, has significantly reduced efficacy at standard dosing in hemodialysis patients and particularly in kidney transplant recipients. Nevertheless, a significant proportion of patients achieved protective antibody titers with excellent safety outcomes. Kidney transplant recipients taking mycophenolate and those with poor allograft function had the lowest likelihood of achieving seroprotection. Repeat vaccination increased seroprotection rates, supporting the current recommendation for annual influenza vaccination of renal transplant recipients.

**MATERIALS AND METHODS**

**Study design and setting**

This is a prospective cohort study, assessing seroresponses to the monovalent pH1N1 vaccine in kidney transplant recipients and hemodialysis patients within the Department of Nephrology, Monash Medical Centre, Melbourne, Victoria, Australia.

**Study population**

From October to December 2009, we attempted to contact all kidney transplant recipients \( n = 526 \) by post and/or phone or to offer vaccination with the monovalent pH1N1 vaccine (Figure 1). All kidney transplant recipients were potentially eligible for inclusion in the study. Subjects were excluded if they had received the monovalent pH1N1 vaccine previously, had been transplanted <21 days before vaccination, or had a known allergy to the influenza vaccine, eggs, and/or chicken products. All hemodialysis patients treated in two community dialysis facilities were also approached and offered vaccination with the monovalent pH1N1 vaccine \( n = 82 \). Pre- and post-vaccination sera from a cohort of 30 healthy controls who received the monovalent pH1N1 vaccine were kindly provided by CSL Limited (Parkville, Victoria, Australia) and served as a comparison set of samples to sera from kidney transplant recipients and hemodialysis patients. Patients consented to receive the monovalent pH1N1 vaccine, and their de-identified data were used to audit seroresponses.

For the boosting study, we recontacted all our kidney transplant recipients in May 2010 and recommended that they receive the 2010 trivalent influenza vaccine. Of the original 151 transplant recipients, 49 who received the monovalent pH1N1 vaccine received the 2010 trivalent influenza vaccine and had their antibody titers repeated.

**Data collection and measures**

Patients attended outpatient vaccination clinics wherein they received a single 15 µg intramuscular dose of the monovalent unadjuvanted, split-virus pH1N1 vaccine (Panvax H1N1, CSL Biotherapies, Parkville, Victoria, Australia). Pre- and 1 month post-vaccination sera were collected. Kidney transplant recipients in the boosting study received a single intramuscular dose of a 2010 trivalent inactivated unadjuvanted split-virus vaccine containing 15 µg of A/California/7/2009 (H1N1-like) antigen and had antibody titers measured at a median of 56.5 days (range 27–178 days) post vaccination.

Antibody titers for pH1N1 were measured using hemagglutination inhibition assays at the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne, using methods described previously. Briefly, sera were pretreated by combining 1 part of serum with 4 parts of receptor-destroying enzyme II (Denka Seiken, Tokyo, Japan) (volume/volume) and incubation at 37 °C for 12 h, followed by the addition of 5 parts of 1.6% sodium citrate and incubation at 56 °C for 30 min. Egg-grown A/California/7/2009 virus was purified by sucrose gradient, concentrated and inactivated with β-propiolaceton (kindly provided by CSL Limited), and diluted to 4 hemagglutinating units/25 µl. Following a 1-h incubation of virus with antisera, 25 µl of 1% (volume/volume) turkey red blood cells was added to each well, and the hemagglutination inhibition test was read after 30 min of incubation. Titers were expressed as the reciprocal of the highest dilution of serum where hemagglutination was prevented.

Subject demographics, comorbidity, cause of end-stage kidney disease, and medication use including current and previous immunosuppressive drug regimen was assessed at the time of vaccination. Kidney function was assessed by the eGFR, calculated using the 4-variable IDMS traceable MDRD study equation. Serum 25-hydroxy vitamin D levels were also obtained before vaccination.

**Study outcomes**

Seroprotection was defined as having an anti-pH1N1 titer of ≥40. Seroconversion was defined as having an anti-pH1N1 titer of ≥40 and a rise in antibody titer of ≥4 times the pre-vaccination level.

**Statistical methods**

Univariate associations between antibody responses in the three groups were explored using analysis of variance or Kruskal–Wallis rank test for continuous variables and the \( \chi^2 \) test or Fisher’s exact test for categorical variables where appropriate. Geometric means of antibody titers were also calculated (sera with a titer of <10 were recorded as 5).

Multivariate logistic regression was used to assess factors associated with an increased likelihood of seroconversion or seroprotection following vaccination with the monovalent pH1N1 vaccine, including female gender, subject age, hemodialysis or kidney transplant recipient, kidney transplant age, immunosuppressive regimen (calcineurin inhibitor, mycophenolate, steroid, and rituximab use), and transplant function. Models were built using backward stepwise elimination of covariates, using Wald tests and a threshold of 0.10 for retention. Any clinically important factors were included in the final model.
(regardless of statistical significance) were kept in the final model. We declared a finding to be statistically significant if the two-sided p-value was < 0.05. All analyses were conducted on Stata 11.2 (StataCorp, College Station, TX).

DISCLOSURE
This study and manuscript preparation received no funding from, nor was in any way influenced by, a commercial organization. The Melbourne WHO Collaborating Centre for Reference and Research on Influenza is supported by the Australian Government Department of Health and Ageing. All the authors declared no competing interests.

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