Reduced incidence of pneumonia in influenza-vaccinated solid organ transplant recipients with influenza disease

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Abstract

Whether influenza vaccination influences the severity of illness in cases of clinical failure in solid organ transplant (SOT) recipients receiving influenza vaccine has not been extensively studied. Our goal was to evaluate the frequency of influenza vaccination among SOT recipients with influenza disease and its impact on the illness severity during the 2010–2011 season. Adult SOT recipients with confirmed influenza infection were included from December 2010 to April 2011. Follow-up data were recorded and antibody titres were determined using a microneutralization assay. Sixty-four SOT recipients were included in the study, ten (15.6%) with severe disease, requiring admission to intensive care units, of whom four (6.3%) died. In all, 34 (53.1%) received the 2010–2011 seasonal influenza vaccine and 32 (50.0%) received the 2009-H1N1 pandemic vaccine, and none had detectable antibodies against influenza at the time of diagnosis of influenza infection. Twenty-three (67.6%) of the patients that received the vaccine required hospital admission and presented less dyspnoea (10, 29.4% versus 14 (50.0%), p 0.09) and pneumonia (8, 23.8% versus 15, 50.0%, p 0.03, relative risk 0.3, 95% CI 0.1–0.9) than unvaccinated patients, with relative risk reductions of 60% and 70%, respectively. Although influenza vaccination confers protection on SOT recipients against developing influenza pneumonia, the rate of clinical failure is still high. New strategies to improve influenza immunization are needed for this group of patients.

Keywords: Influenza immunization, influenza infection, pneumonia, seasonal influenza vaccine, solid organ transplantation
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Introduction

Respiratory infections, especially influenza infections, are a major cause of morbidity and mortality in solid organ transplant (SOT) recipients [1]. Influenza infections cause complications including primary and secondary viral pneumonia [1,2] and prolonged periods of viral shedding [3]. Since the last influenza virus A (H1N1) pandemic in 2009, an increased knowledge of influenza infection has confirmed the severity of the infection in this population. Different studies in SOT recipients have reported a rate of mortality associated with influenza infection ranging from 4% to 8%, with severe cases or complications in 12–20% of cases [1,4].

Annual vaccination for seasonal influenza is the most effective strategy for reducing the incidence and complications of influenza infection, which have been shown to reduce mortality and decrease the risk of graft loss in the transplant

population as observed in a retrospective study of 51 730 adults transplanted when vaccinated within the first year after transplantation [5]. However, the response to influenza vaccination in the transplant setting is discordant, with lower efficacy than in the general population with a seroprotection rate that varies between 15% and 90% [6–16]. Suboptimal vaccination response may be partly a result of the incomplete protection against influenza reported for vaccination in high-risk individuals [17]. Older individuals appear to be at particular risk for vaccine failure, perhaps as a result of immune senescence [18].

Clinical failure of influenza vaccination in SOT recipients has not been extensively studied. A retrospective cohort study, which evaluated the clinical effectiveness of the 2009-adjuvanted pandemic influenza vaccine in 168 patients, showed that 96.4% were seroprotected [19]. In a multicentre study mentioned previously, we demonstrated a rate of clinical failure after receiving one dose of the pandemic vaccine of 1.1% [20]. However, there are no clinical studies that evaluate the impact of the antecedent of influenza vaccination in the SOT recipients with influenza disease. Hence, the goal of the present study was to evaluate the frequency of non-adjuvanted 2010–2011 seasonal influenza vaccination among SOT recipients with influenza disease and its repercussion on the illness severity.

Materials and Methods

Subjects and study design

This prospective cohort observational study was conducted at eight teaching hospitals belonging to the Spanish Network for Research in Infectious Diseases (REIPI). All SOT recipients older than 16 years diagnosed with confirmed influenza virus infection who attended hospital from December 2010 to April 2011 were included. Cases were detected on a daily basis by reviewing the microbiological reports. A confirmed case was defined as the presence of influenza-like illness with laboratory-confirmed influenza infection. The study was approved by the local ethics committee and informed consents were obtained from all subjects. This study was carried out considering the current legislation, the ethical regulations of the Helsinki Declaration and the guidelines on good clinical laboratory practice.

Clinical parameters

Patients were advised to seek care at the hospital in cases of respiratory symptoms. One or more of the investigators in each participating hospital evaluated and followed patients after influenza diagnosis, and clinical data were recorded in a standardized, computer-assisted protocol. Data were collected on demographic characteristics, co-morbidities, body mass index, type of transplant and immunosuppressive therapy, time since transplantation, previous history of vaccination, clinical signs and symptoms, biochemical analysis, chest X-ray findings, antiviral and antibacterial therapy, concomitant and secondary bacterial infections, time to clinical stability, and outcomes, including mortality. A follow-up visit took place 28 days after diagnosis. Completed protocols were revised by a senior investigator before the final validation.

Cases of influenza A (2009-H1N1, H3N2) infection were diagnosed by RT-PCR (Inf A/H1N1 Detection; Roche, Mannheim, Germany) and influenza B virus was detected using the Detection Set, Lightcycler 2.0 system, (Roche; for details, see Supplementary material, Data S1). Other respiratory viral infections were tested by multiplex-PCR (mPCR; Seeplex-RV15ACE Detection kit, Seegene Inc., Seoul, Korea) that detects 15 respiratory viruses (human metapneumovirus, adenovirus, coronavirus 229E/NL63, coronavirus-OC43/HKU1, parainfluenza 1–3, rhinovirus A/B/C, respiratory syncytial A-B and influenza A-B, enterovirus, bocavirus 1/2/3/4). Concomitant and secondary non-viral infection (co-infection) was considered if a bacterium or fungus was isolated from at least one of the following samples: blood, pleural fluid, sputum culture, bronchial aspirate, bronchoalveolar lavage, protected specimen brush, transbronchial biopsy or in cases with positive urinary antigen tests for *Streptococcus pneumoniae* and *Legionella pneumophila* serogroup 1. Hypogammaglobulinaemia was defined as IgG levels under 700 mg/dL. Pneumonia was defined by the presence of clinical symptoms (fever, dyspnoea, cough and expectoration) and a new pulmonary infiltrate in the chest X-ray for which other non-infectious causes were excluded. Severe disease was defined as requiring admission to intensive care (respiratory insufficiency, septic shock and severe sepsis), mortality during influenza illness or allograft rejection. Early antiviral therapy was defined as the administration of antiviral agents active against 2009 influenza A/H1N1 or B within the 48 h after the onset of symptoms.

Vaccination

Recipients of SOT received the influenza seasonal vaccine at their healthcare centres as part of the national vaccination recommendations [21]. Vaccinated patients were given one dose of the trivalent non-adjuvanted vaccine for the 2010–2011 influenza season (Gripavac; Sanofi-Pasteur MSD, Madrid, Spain). Each dose of vaccine contained the strains included in the trivalent seasonal influenza 2010–2011 vaccine: A/California/7/2009(H1N1) (2009-H1N1 strain), A/Perth/16/2009(H3N2) (H3N2 strain) and B/Brisbane/60/

2008-like (B strain). Patients were considered to be vaccinated against influenza viruses if the vaccine was administered at least 6 weeks before influenza infection.

Microneutralization assay

Antibody titres for the influenza 2009-H1N1 strain were determined using a microneutralization assay as previously described [20], (for details, see Supplementary material, Data S1).

Statistical analysis

The results were analysed using the statistical software package (SPSS, version 15.0; SPSS Inc, Chicago, IL). A descriptive statistical analysis was performed. All proportions were calculated as percentages of the patients with available data and median and interquartile range (IQR) for continuous variables. We identified clinical manifestations and risk factors of severe disease in all SOT recipients with influenza infection and we measured the primary effectiveness of influenza vaccination in reducing pneumonia or severe disease incidence by comparing the proportions of these between vaccinated and unvaccinated patients in the follow-up cohort, which we calculated as (1 – relative risk) × 100. To detect significant differences between groups, we used the chi-square test or Fisher exact test for categorical variables and the *t* test, Mann–Whitney *U* test and Wilcoxon test for continuous variables, when appropriate. For the multivariate analysis, logistic regression was used for factors influencing the risk of pneumonia. The relative risks (RR) and 95% CI of geometric mean titres (GMT) were calculated by taking the exponent of natural logarithm of the mean and 95% CI. Statistical significance was established at $\alpha = 0.05$. All reported p values are two-tailed.

Results

A total of 64 SOT recipients were included, of those, 47 (73.4%) patients required hospital admission. Types of transplant were 32 (50.0%) kidney, 14 (21.9%) liver, 14 (21.9%) heart and four (6.3%) lung with a median time from transplantation of 3.8 years (1.7–8.6 years). In one vaccinated heart transplant patient, influenza occurred 12 days after transplant. Most patients (59, 92.2%) were diagnosed with influenza A H1N1 (2009) virus and only five cases (7.8%) were diagnosed with influenza B virus infection.

The median age was 59 years (48.2–65.0 years) and 44 (68.8%) were men. Fifty SOT recipients (78.1%) had comorbid conditions other than transplantation, with high frequencies of chronic kidney and heart disease (29, 45.3% and 22, 34.4%, respectively). The most common immunosuppressive drugs were tacrolimus (38, 59.4%), mycophenolate mophetil (50, 78.1%) and corticosteroids (41, 64.1%) in a triple combined therapy. Thirty-four (53.1%) patients received the 2010–2011 seasonal influenza vaccine more than 5 weeks before infection and 32 (50.0%) had received the pandemic influenza A H1N1 (2009) vaccine the previous season (Table 1).

Clinical manifestations and outcomes: risk factors of severe disease

The median time from symptom onset to influenza diagnosis was 4.5 days (IQR 2.0–10.0) with the most common manifestations being cough (56, 87.5%) and fever (48, 48.4%; Table 2). Ten (15.6%) patients had severe disease and pneumonia, requiring admission to intensive care and four (6.3%) of them died. No other respiratory viruses were detected in the samples. Bacterial co-infection was more common in patients with severe disease (3, 30% versus 4, 7.4%, p 0.03, RR 3.4, 95% CI 1.1–10.5) with *S. pneumoniae* (two cases) and *Aspergillus* spp. (one case) in patients with severe disease versus *S. pneumoniae* (one case), *Pseudomonas aeruginosa* (two cases) and *Aspergillus* spp. and *Rhizopus* spp. (one case) in patients with non-severe disease.

Other factors associated with severe disease were: chronic heart disease (7, 70% versus 15, 24.8%, p 0.01, RR 4.5, 95% CI 1.3–15.6), chronic pulmonary disease (5, 50% versus 11, 20.4%, p 0.04, RR 3, 95% CI 1–9), dyspnoea (7, 77.8% versus 17, 32.1, p 0.009, RR 5.5, 95% CI 1.2–24.5) and pneumonia (10, 100% versus 13, 24.1%, p <0.001; Table 3).

Patients with severe disease initiated antiviral therapy at a median of 10.5 days (3.8–15.8 days) and patients with non-severe disease at a median of 4 days (3–11 days) (p 0.21). Two patients with mild disease (3.1%) did not receive oseltamivir therapy, having favourable outcome. Median time of antiviral therapy was 10 days (6.5–14.0 days) versus 5 days (5.0–7.0 days; p 0.002) in severe and mild cases, respectively.

Immunological response to vaccination and clinical repercussions

In 44 (68.7%) patients antibody titres against influenza A H1N1 (2009) or influenza B virus were measured at the time of detecting the infection and 4 weeks later. At the moment of influenza infection none of the patients, including 34 patients who had received 2010–2011 seasonal influenza vaccine, had detectable antibody titres against influenza viruses. At a median of 28 days after the influenza infection symptom onset, 20 (45.5%) patients had developed antibody titres with a GMT post-infection of 144.2 (95% CI 113.2–183.6). Seroprotection after influenza infection happened more frequently in patients with pneumonia (9, 45.0% versus 2,

TABLE 1. Patient characteristics according to the status of the 2010–2011 seasonal influenza vaccination

Baseline characteristics	Total (*n* = 64)	Vaccinated (*n* = 34)	Unvaccinated (*n* = 30)	p
Sex				
Male	44 (68.8%)	24 (70.6%)	20 (60.7%)	0.73
Age (median, IQR)	59 (48.2–65.0)	60.5 (48.7–65.7)	58 (48–65)	0.44
≤65 years	47 (73.9%)	25 (73.5%)	22 (73.3%)	0.98
>65 years	17 (26.6%)	9 (26.5%)	8 (26.7%)	
Type of transplant				
Kidney	32 (50%)	26 (47.1%)	16 (53.3%)	0.21
Liver	14 (21.9%)	5 (14.7%)	9 (30%)	
Heart	14 (21.9%)	10 (29.4%)	4 (13.3%)	
Lung	4 (6.3%)	3 (8.8%)	1 (3.3%)	
Comorbidities	50 (78.1%)	27 (79.4%)	23 (76.7%)	0.79
Chronic pulmonary disease	16 (25%)	9 (26.5%)	7 (23.3%)	0.77
Chronic heart disease	22 (34.4%)	12 (35.3%)	10 (33.3%)	0.86
Diabetes mellitus	15 (23.4%)	12 (35.3%)	3 (10%)	0.01
Chronic kidney disease	29 (45.3%)	15 (44.1%)	14 (46.7%)	0.83
Chronic liver disease	8 (12.5%)	4 (11.8%)	4 (13.3%)	0.85
Influenza vaccines				
2010–2011 vaccine	34 (53.1%)			
2009–2010 vaccine	38 (59.4%)	31 (93.3%)	7 (28%)	<0.001
2009 H1N1	32 (50%)	27 (87.1%)	5 (20%)	<0.001
Hypogammaglobulinaemia	10 (15.6%)	5 (14.7%)	5 (17.9%)	0.73
Immunosuppressive therapy				
Cyclosporine	17 (26.6%)	9 (26.5%)	8 (26.7%)	0.98
Tacrolimus	38 (59.4%)	19 (55.9%)	19 (63.3%)	0.54
MMF	50 (78.1%)	23 (67.6%)	27 (90%)	0.03
mTOR inhibitors	12 (18.8%)	9 (26.5%)	3 (10%)	0.09
Corticosteroids	41 (64.1%)	20 (58.8%)	21 (70%)	0.35
Monoclonal antibody induction therapy	5 (7.8%)	2 (5.9%)	3 (10%)	0.54

IQR, interquartile range; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin.
Parameters were compared statistically by Chi-square test, p ≤0.05 was considered significantly different. The Mann–Whitney *U* test was used for continuous variables.

TABLE 2. Clinical characteristics and outcomes in vaccinated versus unvaccinated patients

	Total (*n* = 64)	Vaccinated (*n* = 34)	Unvaccinated (*n* = 30)	p
Hospitalization	47 (73.4%)	23 (67.6%)	24 (80%)	0.26
Clinical symptoms				
Rhinorrhoea	30 (46.9%)	19 (55.9%)	11 (39.3%)	0.19
Cough	56 (87.5%)	29 (85.3%)	27 (96.4%)	0.14
Fever	31 (48.4%)	15 (44.1%)	16 (57.1%)	0.30
Dyspnoea	24 (37.5%)	10 (29.4%)	14 (50%)	0.09
Diarrhoea	13 (20.3%)	10 (29.4%)	3 (10.7%)	0.07
Myalgia-arthralgia	20 (31.3%)	12 (35.3%)	8 (28.6%)	0.57
Laboratory and radiological findings				
Leucopenia (<4000/mm)	10 (15.6%)	7 (20.6%)	3 (10.3%)	0.26
Leucocytosis (>12 000/mm)	6 (9.4%)	3 (8.8%)	3 (10.3%)	0.83
Neutropenia (<500/μL)	3 (4.7%)	2 (5.9%)	1 (3.6%)	0.67
Lymphopenia (<1500/μL)	47 (73.4%)	21 (61.8%)	26 (89.7%)	0.01
Anaemia (haematocrit <36%)	34 (53.1%)	16 (47.1%)	18 (62.1%)	0.23
Thrombocytopenia (<150 × 10^3/μL)	20 (31.3%)	11 (32.4%)	9 (31%)	0.91
Pneumonia	23 (35.6%)	8 (23.5%)	15 (50%)	0.02
Bacterial co-infection	7 (10.9%)	4 (11.8%)	3 (10%)	0.82
Clinical outcomes				
Severe disease	10 (15.6%)	4 (11.8%)	6 (20%)	0.36
ICU admission	10 (15.6%)	4 (11.8%)	6 (20%)	0.36
Death	4 (6.3%)	2 (6.7%)	2 (5.9%)	0.89
Length of hospital stay (median, IQR)	12 (7.0–29.0)	8 (5.0–18.0)	12.5 (8.0–31.0)	0.03

ICU, intensive-care unit admission; IQR, interquartile range.
Parameters were compared statistically by Chi-square test, p ≤0.05 was considered significantly different. The Mann–Whitney *U* test was used for continuous variables.

8.3%, RR 2.4, 95% CI 1.4–4.3, p 0.005) and severe disease (5, 25.0% versus 0, 0.0%, RR 2.6, 95% CI 1.7–3.6, p 0.009). In addition, GMT post-infection were also higher in cases of pneumonia (63.6, 95% CI 15.3–264.5 versus 5.1, 95% CI 2.2–11.7 p 0.007) and in severe disease (211.1, 95% CI 97.8–455.9 versus 6.5, 95% CI 2.9–14.0; p 0.001). Seroprotection rate or GMT post-infection were not related with receiving 2010–2011 seasonal influenza vaccine (55.0% versus 58.3%; p 0.82 and 8.6, 95% CI 3.1–24.0 versus 11.1, 95% CI 3.1–39.4; p 0.65, respectively). In two of the patients who died (4.5%) antibody titres after the infection could be determined with a GMT post-infection of 160 and 320, respectively.

Twenty-three (67.6%) of the 34 patients that received the 2010–2011 seasonal influenza vaccine required hospital admission. Vaccinated patients less frequently experienced

TABLE 3. Characteristics of patients with severe disease

	Severe disease (*n* = 10)	Non-severe disease (*n* = 54)	p
Age			
≤65 years	7 (70%)	40 (74.1%)	0.78
>65 years	3 (30%)	14 (26%)	
Time since transplantation (years)	6.1 (0.19–16.8)	3.9 (0.07–22.9)	0.41
Type of transplant			
Kidney	6 (60%)	26 (48.1%)	0.73
Liver	2 (20%)	12 (22.2%)	
Heart	1 (10%)	13 (24.1%)	
Lung	1 (10)	8 (15.4%)	
Immunosuppressive therapy			
Cyclosporine	2 (20%)	15 (27.8%)	0.60
Tacrolimus	7 (70%)	31 (57.4%)	0.45
MMF	9 (90%)	41 (75.9%)	0.32
mTOR inhibitors	1 (10%)	11 (20.4%)	0.44
Corticosteroids	8 (80%)	33 (61.1%)	0.25
Monoclonal antibody induction therapy	0 (0%)	5 (9.3%)	0.31
2010–2011 seasonal influenza vaccination	4 (40%)	30 (55.6%)	0.36
Hypogammaglobulinaemia	2 (20%)	8 (15.4%)	0.71
Comorbidities	9 (90%)	41 (75.4%)	0.32
Chronic pulmonary disease	5 (50%)	11 (20.4%)	0.04
Diabetes	0 (0%)	15 (27.8%)	0.06
Chronic heart disease	7 (70%)	15 (27.8%)	0.01
Chronic renal failure	6 (60%)	23 (42.6%)	0.31
Chronic liver failure	3 (30%)	5 (9.3)	0.07
Clinical variables			
Rhinorrhoea	2 (22.2%)	28 (52.8%)	0.09
Cough	9 (90%)	47 (87%)	0.28
Dyspnoea	7 (77.8%)	17 (32.1%)	0.009
Diarrhoea	2 (22.2%)	11 (20.8)	0.92
Myalgia-arthralgia	1 (10%)	19 (35.2%)	0.14
Pneumonia	10 (100%)	13 (24.1%)	<0.001
Time to initiation of treatment	10.5 (3.8–15.8)	4 (3.0–11.0)	0.21
Bacterial co-infection	3 (30%)	4 (7.4%)	0.03

MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin.
Parameters were compared statistically by Chi-square test, p ≤0.05 was considered significantly different. The Mann–Whitney *U* test was used for continuous variables.

dyspnoea (10, 29.4% versus 14, 50.0%, p 0.09) with a relative risk reduction of 60.0% (95% CI 37.1–88.3%) and pneumonia (8, 23.8% versus 15, 50.0%, p 0.03, RR 0.3, 95% CI 0.1–0.9) with a relative risk reduction of pneumonia of 70% (95% CI 42.2–97.6%). Another factor associated with pneumonia was chronic pulmonary disease. In the multivariate analysis, receiving the influenza vaccine 2010–2011 was the only modifiable independent associated factor for pneumonia (OR 0.15, 95% CI 0.03–0.62, p 0.009; Table 4).

Four vaccinated patients and six unvaccinated had severe disease (11.8% versus 20.0%, p 0.36), all of them with pneumonia and requiring admission to intensive care. Two patients in each group died (2, 5.9% versus 2, 6.7%, p 0.89). The proportion of patients receiving early antiviral therapy was similar in vaccinated and unvaccinated patients (23.3% versus 18.5%; p 0.65). The median length of hospital stay was different: 8 days (5.0–18.0 days) in vaccinated patients, and 12.5 days (8.0–31.0) in unvaccinated patients, p 0.03; Tables 2 and 3).

Discussion

Our results show that the antecedent of seasonal vaccination must not preclude the consideration of influenza infection in SOT recipients with respiratory symptoms in epidemic periods. Hence, more than half of patients with influenza illness had received 2010–2011 influenza vaccination, and most of them required hospital admission, with a severe disease occurring in 12% of them. However, influenza vaccination was associated with a lower incidence of pneumonia and shorter length of hospital stay.

Since the last pandemic it has been demonstrated that influenza infection in SOT recipients is associated with a higher mortality rate than in the general population [1,4]. The mortality of influenza infection in our cohort (6%) was associated with having bacterial co-infections at the moment of initiating antiviral treatment and with other prognostic factors previously described such as the presence of comorbidities (chronic disease), time of initiating oseltamivir treatment, developing dyspnoea or pneumonia, among others [22–24].

Administration of the annual influenza vaccine is currently the most effective prevention strategy for influenza infection in SOT recipients. Although some studies have shown similar responses to the general population in renal [16] and liver [9] transplant recipients, most of the studies clearly suggest a reduced immune response in kidney [7,10,14,15], liver [8,25] and heart [6,8] recipients. However, to our knowledge this is the first study to analyse the clinical failure of the influenza vaccine in a cohort of SOT recipients with influenza illness and the role of vaccination on the clinical manifestation of influenza infection in this population.

The present study demonstrates a high rate of 2010–2011 seasonal influenza vaccination among SOT recipients with influenza. This is especially relevant, as influenza infection must not be ruled out in influenza-vaccinated SOT recipients with respiratory symptoms or pneumonia in pandemic or epidemic periods. A high index of suspicion and an early diagnosis is mandatory, given the fact that, as previously reported, we found that time from onset of symptoms to initiating oseltamivir therapy was associated with the severity of the illness. Our findings are in agreement with previous studies characterizing the immunological response to influenza vaccine in SOT recipients. Although some of these studies have shown similar responses in renal [16] and liver [9] transplant recipients compared with that of the general population, most of them showed a reduced humoral immune response in renal [7,10,14,15], liver [8,25,26], lung [27] and cardiac [6,8] transplant recipients, with a seroprotection rate that varies between 15% and 90% [6–16] and a

TABLE 4. Multivariate analysis of factors influencing risk of pneumonia

	Bivariate analysis			Multivariate analysis	
	(%) RR (CI 95%)		p	Adjusted OR (CI 95%)	p
Age					
>65 years vs ≤65 years	29.4% vs 38.3%	0.67 (0.17–2.54)	0.51	0.26 (0.04–1.59)	0.14
2010–2011 vaccine					
Yes vs No	23.5% vs 50.0%	0.3 (0.1–0.9)	0.03	0.15 (0.03–0.6)	0.007
Chronic pulmonary disease					
Yes vs No	63.6% vs 30.2%	4.0 (1.0–15.8)	0.04	5.56 (1.1–27.6)	0.03
Chronic heart disease					
Yes vs No	50.0% vs 28.6%	2.5 (0.8–7.3)	0.09	1.1 (0.27–5.13)	0.81
Days from symptoms onset to antiviral therapy					
Yes vs No	7 (3.2–11.7) vs. 4 (3–11)	1.02 (0.95–1.1)	0.46	1.02 (0.92–1.12)	0.64

Parameters were compared statistically in a logistic regression, p ≤0.05 was considered significantly different.

lower seroprotection rate after vaccination of 78% compared with healthy subjects [7].

However, our results suggest that receiving the influenza vaccine confers protection against the development of pneumonia in patients with influenza infection because it was associated with a decreased risk of developing pneumonia and shorter hospital stay. Attenuation of disease severity has been described for other vaccines such as the 23-valent polysaccharide vaccine against *S. pneumoniae* that was tested in the elderly, where vaccination was associated with a reduction of risk of death due to pneumonia complications [28,29]. Limited data of a similar effect are available for non-replicating influenza vaccines [30,31], but no studies have been performed in transplant recipients. Reduction in illness severity, even among those who are not protected against infection, would enhance the population health benefits of this vaccine.

In the present study, nearly half of the patients had not received influenza vaccination. Vaccination is indicated beyond the second month of transplant in all protocols at all participating centres and this percentage does not represent the proportion of patients that receive vaccination in our SOT recipient population. Nevertheless, an effort should be made to increase the number of patients and relatives receiving influenza vaccination.

None of the patients had antibody titres in response to vaccination at the onset of illness and less than half of them had seroconverted 4 weeks after influenza diagnosis. However, patients that developed severe symptoms of influenza infection presented higher antibody titres at convalescence, probably as a result of the exposure to an increased influenza viral load triggering a stronger immune response.

Our study has several limitations. First, the number of patients included in the study may be underpowered to demonstrate the association of the mortality rate and previous vaccination. In addition, although all patients were advised to seek care at the hospital if they had respiratory tract infection symptoms, it is possible that some patients with mild influenza infection did not attend the hospital. However, all severe cases of influenza were included in the study. As the objective of the study was not to evaluate the clinical efficacy of the vaccine, but to determine vaccination frequency among clinical cases of influenza, the loss of some mild cases does not invalidate our results, which show that vaccination failed in half of SOT recipients with severe influenza. Finally, given the association of severity and seroconversion, the episode of respiratory illness could be caused by other microorganisms and the detection of influenza virus might be related to an asymptomatic colonization. However, although investigated, no other viral co-infections were detected in these patients and the positive predictive value of respiratory symptoms during the influenza epidemic period is very high [32]. We therefore think that these episodes corresponded to true influenza infections.

In conclusion, the response of SOT recipients to influenza vaccine is not optimal with a high rate of failure among hospital-treated influenza cases, demonstrating that new strategies to improve influenza immunization are needed in this patient subgroup. However, influenza vaccination may confer protection against the development of influenza pneumonia. Further studies are warranted to validate these findings in a larger cohort and to evaluate its impact on other outcome endpoints, such as mortality.

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Authors' Contribution

EC provided patient care and designed the research and was responsible for the project; TAA was responsible for collecting patient samples, analysed results and generated all the tables and the paper; AP-O performed the experiments; JT-C, RL and CS provided patient care and participated in clinical data collection; JP participated in the research design and provided patient care; PP-R designed the research and was responsible for the project and the preparation of the paper All authors reviewed the paper and had access to the primary clinical data. Other authors in the SOTR study group (REIPI): From University Hospital Virgen del Rocío. Magdalena Sánchez, Miguel Angel Gentil, Miguel Angel Gómez-Bravo, Ernesto Lage. From Hospital General Universitario Gregorio Marañón. Maddallena Gianella; From Hospital Clinic de Barcelona. Mª Angeles Marcos, Irma Hoyo, Carlos Cervera y Tomás Pumarola. From Hospital Universitario 12 de Octubre. José María Aguado; From Hospital Universitario de Bellvitge-IDIBELL. Núria Fernández-Sabé, Jordi Carratalà; From Hospital Universitario de Cruces. From Hospital Vall d'Hebron. Oscar Len, Evelyn Cabral; From Hospital Universitario Marqués de Valdecilla. Maria Victoria San Juan, Manuel Gutierrez-Cuadra.

Transparency declaration

Authors declare no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Materials and methods.

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