

Effect of annual influenza immunization on antibody response in lung transplant patients

Background—Influenza viral infections cause significant morbidity and mortality each season. Lung transplant patients may be at higher risk because of their underlying pathophysiology. Although annual immunization is the standard of care, its efficacy remains largely unproven. Previous studies showed poor antibody response to influenza vaccine in lung transplant patients, but no data on the antibody response in consecutive seasons have been published.

Methods—We studied antibody responses to influenza vaccine in 122 subjects: 66 lung transplant recipients, 28 control subjects, and 28 patients awaiting lung transplantation. We compared antibody response rates to individual viruses contained in influenza vaccines in consecutive years within the 3 groups. Serum antibody concentrations were measured at baseline and 2 to 4 weeks after vaccination by using the hemagglutination inhibition assay. Log-transformed antibody concentrations and incidence of seroconversion and seroprotection were calculated.

Results—Median log-transformed antibody responses were similar in consecutive seasons in lung transplant subjects. Incidences of seroprotection and seroconversion did not differ between consecutive seasons in lung transplant recipients.

Conclusions—Antibody responses were similar in consecutively measured years in lung transplant subjects. Annual influenza vaccination in lung transplant subjects produces similar immune responses in 2 consecutive years, indicating that these patients are not at significantly increased risk of vaccine failure. (*Progress in Transplantation*. 2009;19:153-159)

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Lung transplant patients may be at an incrementally higher risk of influenza infection than other transplant patients. Factors that predispose them to respiratory infection include high degree of immunosuppression, altered mucociliary clearance, repeated airway instrumentation, bronchial anastomotic obstruction, disruption of lymphatic drainage, and the exposure of the transplanted organ to the environment.¹

The influenza vaccine used for those at high risk for influenza morbidity and mortality is an inactivated trivalent preparation. Influenza vaccine contains 2 type A viruses and 1 type B virus. The vaccine is reformulated each year based on the viruses expected to circulate during the upcoming season. A protective

antibody response to influenza vaccine is considered an antibody titer of at least 1:40. Seroconversion following influenza immunization implies at least a 4-fold increase in antibody concentrations.^{2,3} Although these indicators of a positive response to influenza immunization have significant overlap, they are not exactly the same. One must consider that a person may have antibodies before immunization because of previous exposure to a vaccine virus or an antigenically similar influenza strain.

Seroconversion rates following influenza vaccination are lower in lung transplant patients than in healthy persons.^{4,5} Clearly, immunization elicits an antibody response in this population, but the response is not

as vigorous as in immunocompetent persons. We previously demonstrated that only the B/HongKong virus was associated with a lower seroconversion rate, and this virus was the only one to change from the previous year's vaccine.⁴ Seroconversion rates decrease with subsequent exposure to the same influenza vaccine viruses, but seroprotection rates remain the same.⁶ It is conceivable that the seroconversion response is more affected by immunosuppression than is the seroprotection response. Stated another way, the ability to mount a high spike in antibody production is hindered. It is difficult to fully ascertain the exact degree of immune impairment in lung transplant recipients because both studies investigating influenza vaccine response used healthy persons as the comparison group. Healthy persons may have less experience with influenza vaccine than do persons with severe respiratory conditions. An immunologically naive host is more likely to mount the large change in antibody concentration and increase the likelihood of seroconversion.⁶ Therefore, we also used patients waiting for lung transplantation as a comparison group.

Methods

Subjects

We enrolled 122 subjects (59 men, 63 women) to receive either the 2004-2005 influenza vaccine, the 2005-2006 influenza vaccine, or both. Three groups of subjects were enrolled: patients who had a previous lung transplant, patients who were awaiting lung transplantation, and healthy control subjects without lung disease. We enrolled 66 transplant recipients, 28 pretransplant patients, and 28 healthy control subjects. Lung transplant subjects and pretransplant subjects were recruited out of the University of Wisconsin's lung transplant clinic. Pretransplant participants who received a lung transplant during the study were moved to the lung transplant group. Six patients changed groups after they had provided the postimmunization blood sample for 2004-2005 but before they had provided the preimmunization blood sample for 2005-2006. Control subjects were health care employees at the University of Wisconsin Hospitals and Clinics or volunteers from the community. The studies were approved by the University of Wisconsin Health Sciences Institutional Review Board for Human Investigation, and all participants gave written informed consent.

Protocols and Procedures

This prospective, parallel study was done to investigate the responses to influenza vaccine in consecutive years. All participants had a blood sample taken for measurement of influenza antibody titers before receiving the influenza vaccine. Subsequently, the 2004-2005—A/New Caledonia/20/99(H1N1)-like, A/Wyoming/3/2003(H3N2), and B/Shanghai/361/2002-

like antigens—and/or 2005-2006—A/New Caledonia/20/00(H1N1)-like, A/California/7/2004(H3N2)-like, and B/Shanghai/361/2002-like antigens—influenza vaccine was administered intramuscularly. Serum was stored at -80°C until the day of analysis. A second blood sample was obtained 2 to 4 weeks later to measure antibody response. Antibody responses to influenza vaccination are similar at 2 and 4 weeks.⁷

Antibody Concentrations. Influenza antibody concentrations were measured by hemagglutination inhibition assay (HIA) in samples taken before immunization and 2 to 4 weeks later. The laboratory staff performing HIA by using standard microtiter techniques was blinded to participant status. Briefly, antibodies present in the human serum inhibit agglutination of guinea pig red blood cells induced by influenza virus. Serial dilutions of the sera are made. Titrated influenza antigen is incubated with the serum dilutions for 30 minutes. Guinea pig red blood cells (50 μ L of 0.5% in phosphate buffered saline) are added and incubated for 45 minutes. The dilution of serum that no longer inhibits hemagglutination is the influenza antibody titer. Antibody concentrations that were below the lower limit of detection (<10 hemagglutination units [HAU]) were assigned a concentration of 1 HAU.

Data Analysis. The primary outcome was the log-transformed antibody response compared between the 2004-2005 and 2005-2006 seasons in the lung transplant group. Antibody response is the difference in serum concentrations from before and 2 to 4 weeks after vaccination. We compared antibody concentrations between seasons by using a Wilcoxon rank sum test because of its robustness.⁸ However, our antibody responses were not normally distributed, so this nonparametric test was the best option. We hypothesized we would need 70 lung transplant recipients to observe a 25% difference in mean log-transformed antibody concentration between seasons, assuming standard deviations of 0.54 and 0.38 in the 2004-2005 and 2005-2006 seasons with a power of 0.8.⁴ Seroprotection was defined as an antibody concentration of 40 HAU or greater. Seroconversion was defined as more than a 4-fold increase in serum HAU. We compared seroconversion and seroprotection rates between seasons in all 3 study groups by using χ^2 or Fisher exact tests. We also used χ^2 or Fisher exact tests to compare seroconversion rates in transplant recipients receiving cyclosporine versus tacrolimus immunosuppression. Time since transplant was correlated to antibody responses in the transplant subjects by using a Spearman correlation coefficient to determine the influence of chronic immunosuppression on immune responses. Statistical significance was defined as *P* less than .05.

Table 1 Demographics of subjects

Characteristic	Lung transplant group	Control group	Pretransplant group
No. of subjects	66	28	28
Age, mean (SEM), y	52 (2)	42 (2)	51 (2)
Sex, male/female	32/34	14/14	13/15
Time since transplant, mean (SEM), range, mo	55 (5), 2-156	NA	NA
Received 2004-2005 vaccine	63	26	24
Received 2005-2006 vaccine	50	25	15
Indications for transplantation			
Chronic obstructive pulmonary disease			
2004-2005	22	0	10
2005-2006	18	0	6
Cystic fibrosis			
2004-2005	16	0	1
2005-2006	9	0	1
Alpha-1 antitrypsin deficiency			
2004-2005	6	0	3
2005-2006	6	0	1
Idiopathic pulmonary fibrosis			
2004-2005	8	0	6
2005-2006	9	0	4
Other diseases ^a			
2004-2005	11	0	4
2005-2006	8	0	3

Abbreviations: NA, not applicable; SEM, standard error of the mean.

^a Other diseases: lymphangioleiomyomatosis, primary pulmonary hypertension, congenital heart disease, sarcoidosis, primary ciliary dyskinesia, bronchiectasis.

Results

Demographics of Subjects

Transplant and pretransplant subjects were older than healthy subjects, and the proportion of men to women was similar among all 3 groups (Table 1). The mean (SEM) time since transplant to entry into the study was 55 (5) months for lung transplant recipients. Forty-one transplant recipients were taking cyclosporine as the primary immunosuppressant therapy and 21 transplant subjects were taking tacrolimus.

Antibody Responses

The log-transformed antibody responses induced by the influenza vaccine were similar between the 2004 and 2005 seasons in transplant recipients for all 3 antigens tested (Figure 1). The healthy participants had similar antibody responses to A/New Caledonia in both seasons but showed higher A/H3N2 responses and lower B/Shanghai responses in 2005 than in 2004. In subjects waiting for lung transplantation, antibody responses were significantly reduced in the 2005 season, compared with the 2004 season for the B/Shanghai antigen ($P < .03$; Figure 1).

Incidence of Seroconversion and Seroprotection

Lung transplant participants had similar seroconversion rates to all 3 antigens in the 2004-2005 and

2005-2006 vaccine seasons (Table 2). In parallel with the change in antibody results, healthy control subjects and patients awaiting lung transplantation had higher incidences of seroconversion to B/Shanghai in 2004 than in 2005 ($P < .01$). No differences between seasons were identified for the other vaccine viruses. The rates of seroprotection did not differ between the 2 vaccine seasons in any of the 3 groups. Seroprotection rates for lung transplant subjects are shown in Table 3.

Influence of Immunosuppressive Therapy

The selection of cyclosporine or tacrolimus as a primary immunosuppressant did not influence vaccine responses in transplant recipients. Seroconversion rates were not influenced by use of cyclosporine or tacrolimus as primary immunosuppressant therapy for any of the 3 antigens in the transplant recipients (Figure 2; P was not significant for all). Seroprotection rates were not influenced by immunosuppressant selection for New Caledonia and A/H3N2 antigen. However, seroprotection to B/Shanghai antigen in the 2004-2005 season was greater in subjects receiving tacrolimus than in subjects receiving cyclosporine ($P = .01$). B/Shanghai seroprotection rates were not influenced by immunosuppressive therapy in the 2005-2006 season. Length of immunosuppressive therapy did not predict immune response to any of the 3 antigens tested in transplant

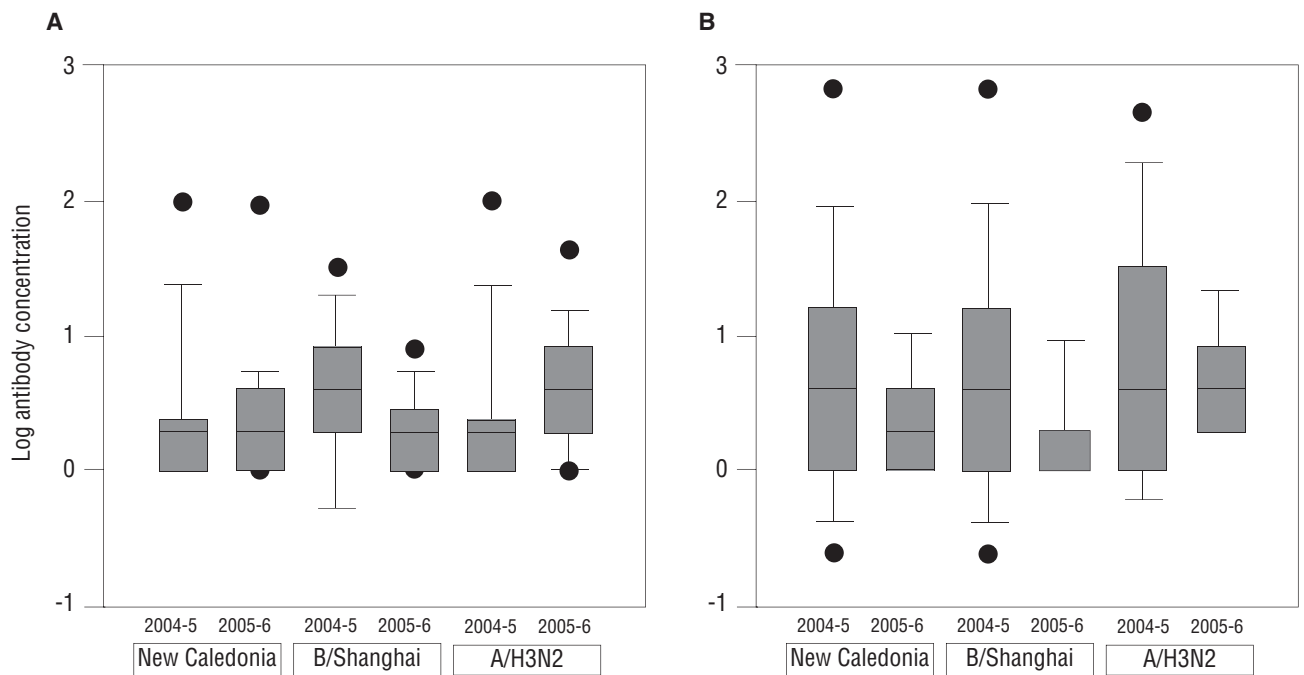


Figure 1 Log-transformed change in antibody responses for control subjects (A), subjects awaiting lung transplantation (B), and lung transplant recipients (C). The change in antibody concentration from before to after immunization are shown for each antigen in the 2004-2005 and 2005-2006 vaccine seasons. Median antibody response to B/Shanghai was lower in the second consecutive year for both healthy persons (median log change in antibody response, 0.6; interquartile range [IQR], 0.3-0.9 in 2004 and median, 0.3; IQR, 0-0.3 in 2005) and patients awaiting lung transplantation (median log change in antibody response, 0.6; IQR, 0-1.2 in 2004 and median, 0; IQR, 0-0.3 in 2005) ($P < .03$ for both).

subjects. Time since transplantation did not correlate with antibody response (Table 4).

Discussion

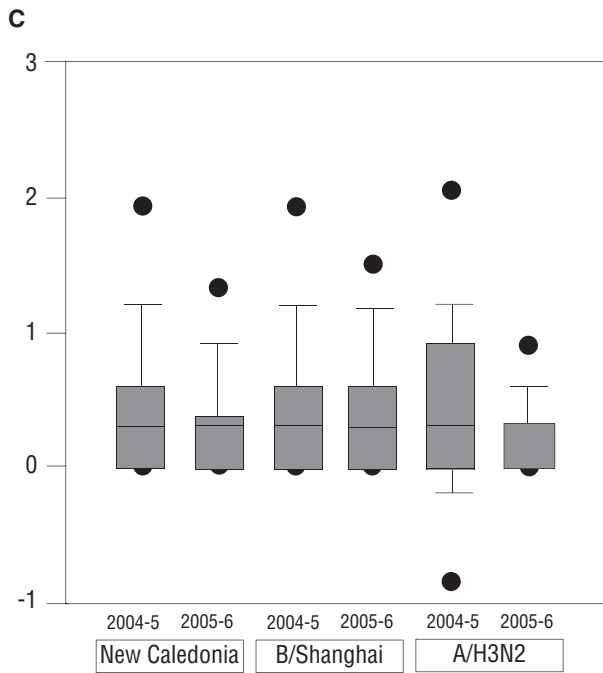
Contrary to our hypothesis, we did not consistently observe lower antibody vaccine responses in lung transplant subjects in the second year of consecutive influenza vaccination. Log-transformed antibody responses were not lower in the second year in lung transplant subjects than in the first vaccination

year. The pattern of responses was slightly different in the lung transplant patients than in the healthy control subjects and pretransplant patients. The H3N2 was a new vaccine virus in 2005, and individuals may mount a more vigorous immune response to the new virus than to influenza viruses that were also included in previous seasons.⁶ The New Caledonia virus had been included in the vaccine for 4 seasons before the initiation of this study, and the B/Shanghai virus was included in just the 2 seasons of this study. Spikes in

Table 2 Incidence of seroconversion to influenza vaccine

Group	Antigen	Incidence of seroconversion		P
		2004-2005	2005-2006	
Lung transplant	New Caledonia	0.31	0.24	.43
	B/Shanghai	0.33	0.22	.18
	A/H3N2 ^a	0.30	0.30	.97
Control	New Caledonia	0.23	0.28	.76
	B/Shanghai	0.62	0.24	.01
	A/H3N2 ^a	0.46	0.64	.26
Pretransplant	New Caledonia	0.54	0.33	.32
	B/Shanghai	0.58	0.13	.008
	A/H3N2 ^a	0.58	0.60	>.99

^a A/Wyoming/3/2003(H3N2) in 2004-2005 vaccine and A/California/7/2004(H3N2)-like in 2005-2006 vaccine.



antibody response decrease with annual exposure to the same influenza vaccine viruses, ⁶ which could explain lower responses to the B/Shanghai vaccine virus in 2005. This phenomenon was not observed for the New Caledonia virus because the subjects may have been repeatedly immunized over the past 6 seasons. The loss of the antibody spike may have occurred before study initiation.

The pretransplant and transplant groups were older than the healthy control subjects, but these differences are not clinically significant from an immunological standpoint. Studies that have results indicating a decreased antibody response to influenza vaccine with age include subjects over age 65 years. ^{9,10}

Because influenza vaccination elicits a lasting but not a permanent immune response, it is conceivable that baseline antibody concentrations in the second year could be higher than in the first year, thus limiting the magnitude of the antibody response. However, baseline median log-transformed antibody concentrations were similar in the 2004-2005 and 2005-2006 seasons for transplant subjects. The baseline antibody concentrations were significantly higher in the 2005-2006 season than in the 2004-2005 season for 1 antigen in control subjects (New Caledonia) and in pretransplant subjects (B/Shanghai). A reduced antibody response was observed for the B/Shanghai antigen in the pretransplant group. Thus, we cannot rule out a ceiling effect of antibody response limiting the antibody response in 2005-2006 in pretransplant subjects for B/Shanghai antigen. We did not witness this phenomenon with the other antigens in pretransplant subjects. Antibody responses in lung transplant subjects were generally smaller than those in the control and pretransplant groups. This would be expected as a general effect from immunosuppressive drug therapy.

Antibody responses were not significantly influenced by different immunosuppressant therapies. Seroconversion rates to all 3 antigens were similar for transplant subjects taking cyclosporine and tacrolimus. However, seroprotection rates to B/Shanghai antigen were significantly higher during the 2004-2005 season in transplant recipients treated with tacrolimus rather than cyclosporine. The clinical significance of this finding is unknown. B/Shanghai and New Caledonia viruses were included in both seasons, but the reexposure low response was not observed with New Caledonia.

Time since transplantation (time being immunosuppressed) did not correlate with log-transformed antibody response consistently, indicating that long-term immunosuppression in lung transplant patients is not predictive of immune response. Immunosuppression protocols are institution-specific, but generally the intensity of immunosuppression decreases with time since transplant as the risk of acute rejection decreases after the first year. Calcineurin inhibitor blood target concentrations are typically lowered as time since

Table 3 Incidence of seroprotection to influenza vaccine in lung transplant recipients

Antigen	Seroprotection before immunization 2004-2005	Incidence of seroprotection 2004-2005	Seroprotection before immunization 2005-2006	Incidence of seroprotection 2005-2006	<i>p</i> ^a
New Caledonia	0.78	0.92	0.88	0.96	.46
BShanghai	0.79	0.81	0.80	0.92	.16
A/H3N2 ^b	0.76	0.94	0.86	0.98	.38

^a χ^2 comparison of incidence of seroprotection between seasons 2004-2005 and 2005-2006.

^b A/Wyoming/3/2003(H3N2) in 2004-2005 vaccine and A/California/7/2004(H3N2)-like in 2005-2006 vaccine.

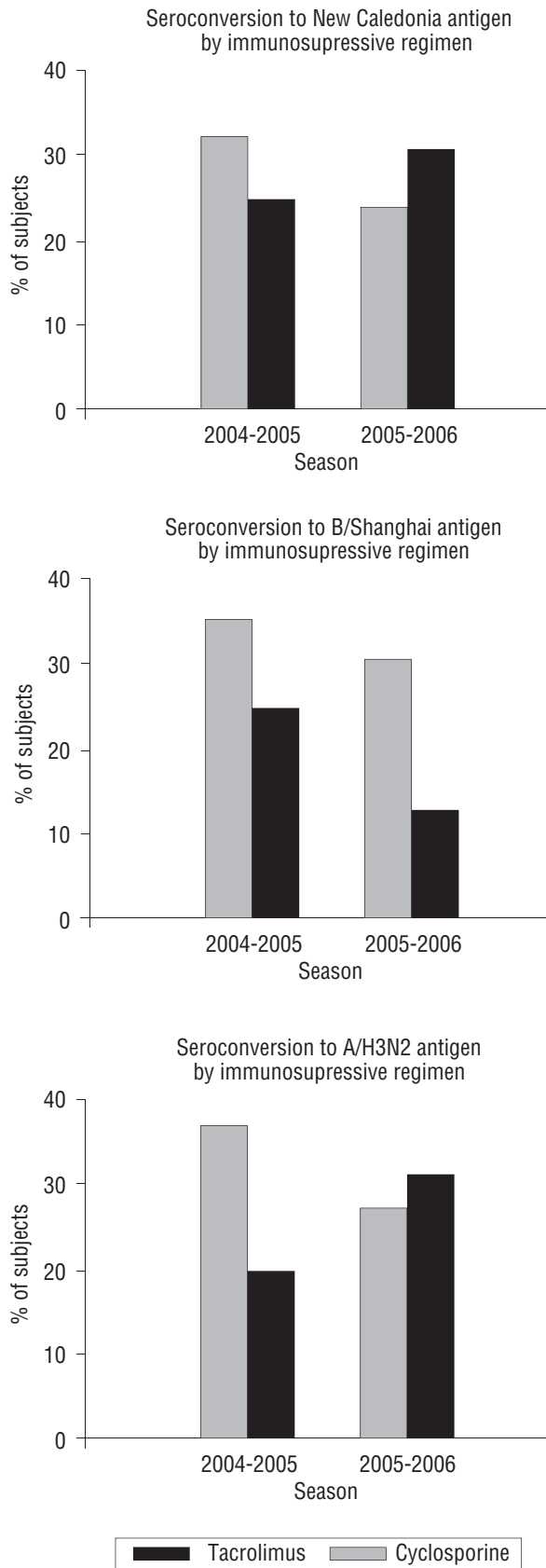


Figure 2 Incidence of seroconversion in patients taking cyclosporine or tacrolimus immunosuppression.

transplantation increases up to 1 or 2 years after transplantation.¹¹ Although a previous study did find an association between cyclosporine therapy and influenza vaccine response rates, no association between cyclosporine blood concentrations and antibody concentrations was found.⁵ Therefore, we used time since transplantation rather than prednisone dose and calcineurin inhibitor trough concentrations for analyses that measured the hypothesized association between immunosuppression intensity and influenza vaccine response.

No impact on vaccine responses could be measured for other immunosuppressive medications. Fifty-eight of 63 and 39 of 50 lung transplant patients in 2004 and 2005, respectively, were taking mycophenolic acid, and 11 of 63 and 10 of 50 were taking sirolimus in these seasons. The small group sizes prohibit making any meaningful comparisons.

The novel finding of this study is that influenza vaccine responses do not significantly wane in consecutive seasons in subjects who have undergone lung transplantation. Our study does have limitations. First, we did not design or analyze our study for differences among our subject groups in vaccine responses. Inclusion of control groups serves to provide a reference point for what occurs in otherwise healthy individuals who have an indication to receive the influenza vaccine. Second, we could not account for exact counts of subjects who received the influenza vaccine in previous seasons before entry into our study. The severe influenza vaccine shortage in 2004-2005 made recruiting healthy persons more difficult. Limited data regarding the antibody concentration needed for protection from influenza in immunosuppressed populations exist so we used thresholds obtained from other populations.^{2,3}

In conclusion, antibody response does not wane during consecutive years of influenza vaccination in lung transplant patients. However, the pattern of response made by lung transplant patients is slightly different from that in nonimmunosuppressed populations. Lung transplant patients were less likely than

Table 4 Correlation of time since transplantation to antibody responses in lung transplant subjects

Antigen	Spearman correlation coefficient	
	2004 season	2005 season
New Caledonia	0.075	0.17
B/Shanghai	0.11	0.041
A/H3N2 ^a	0.075	-0.11

^a A/Wyoming/3/2003(H3N2) in 2004-05 vaccine and A/California/7/2004 (H3N2)-like in 2005-06 vaccine.

healthy persons or patients waiting for lung transplantation to have a significant decrease in seroconversion rates to seasonally repeated vaccine viruses. More research on immune status and responses in this population is needed to address risk of vaccine failure and infectious complications.

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