Immunoglobulin G Levels before and after Lung Transplantation

Natalie H. Yip, David J. Lederer, Steven M. Kawut, Jessie S. Wilt, Frank D'Ovidio, Yuanjia Wang, Edward Dwyer, Joshua R. Sonett, and Selim M. Arcasoy

Departments of Medicine and Surgery, College of Physicians and Surgeons; Department of Epidemiology, Mailman School of Public Health; and Department of Statistics, Columbia University, New York, New York

Rationale: The determinants of immunoglobulin G (IgG) level and the risk of hypogammaglobulinemia (HGG) in patients with severe lung disease before and after lung transplantation are unknown.

Objectives: We aimed to identify predictors of low IgG levels before and after lung transplantation.

Methods: We performed a retrospective cohort study of 40 consecutive lung transplant recipients at our center. Total IgG levels were measured before and serially after transplantation. Mild HGG was defined as IgG levels from 400–699 mg/dl; severe HGG was defined as IgG levels < 400 mg/dl.

Measurements and Main Results: Before transplantation, six (15%) patients had mild HGG, and none had severe HGG. Patients with chronic obstructive pulmonary disease had lower IgG levels compared with patients with other diseases (independent of corticosteroid use and age; $p = 0.001$) and an increased risk of mild HGG ($p = 0.005$). The cumulative incidences of mild and severe HGG significantly increased after transplantation (58 and 15%, respectively, both $p < 0.04$ compared with pretransplant prevalences). Lower pretransplant IgG level and treatment with mycophenolate mofetil were associated with lower IgG levels after transplantation (both $p < 0.05$). Only lower pretransplant IgG levels were significantly associated with an increased risk of severe HGG after transplantation ($p = 0.02$).

Conclusions: Mild HGG is common in patients with severe chronic obstructive pulmonary disease, and the incidences of mild and severe HGG increase significantly early after lung transplantation. Baseline IgG levels and treatment with mycophenolate mofetil affect post-transplant IgG levels.

Keywords: hypogammaglobulinemia; immunosuppression; infection; lung transplantation

Lung transplantation is a therapeutic option for patients with advanced lung disease (1). However, despite recent improvements in short-term outcomes, the 5-yr survival rate remains suboptimal (2). Infections due to bacterial and nonbacterial pathogens result in frequent antibiotic use, hospitalization, and graft dysfunction and account for 26% of all post-transplantation deaths (3). The disproportionate impact of infections in lung transplant recipients compared with that in other solid organ recipients likely results from several factors. Higher levels of immunosuppression; exposure of the lung allograft to the ambient, nonsterile environment and (in single lung transplantation) the native lung; and impairment of the usual microorganism clearance mechanisms contribute to the increased risk of respira-

tory infections in lung compared with other solid organ transplant recipients. Acknowledging the enormous impact of respiratory infection after lung transplantation, the National Institutes of Health has solicited investigation in this area to improve the suboptimal long-term outcomes (4, 5).

We recently demonstrated a high prevalence of hypogammaglobulinemia (HGG) after lung transplantation (6). Patients with severe HGG (IgG level < 400 mg/dl) had a higher cumulative incidence of pneumonia and worse survival. A pretransplant diagnosis of chronic obstructive pulmonary disease (COPD), female sex, and a diagnosis of bronchiolitis obliterans syndrome were significant predictors of severe post-transplant HGG. However, that study was limited by the lack of pretransplant and serial post-transplant IgG levels, making it impossible to calculate the incidence of HGG and analyze detailed time-dependent pharmacologic factors. In the current study, we assessed the determinants of IgG levels and HGG before and early after lung transplantation. Some of these data have been published in abstract form (7).

METHODS

Study Population and Study Variables

We performed a retrospective cohort study of consecutive patients undergoing lung transplantation at New York Presbyterian Hospital between September 2003 and December 2004. None of these patients were included in our previous report. All patients had serum IgG levels measured before and serially after surgery as part of our clinical protocol. If there was more than one assessment preoperatively, the one closest to the time of surgery was used in the analysis. The study was approved by the Columbia University Medical Center Institutional Review Board.

We collected demographic, clinical, and laboratory data from paper and electronic medical records. Patient symptoms, medication use, physical examination data, and clinical events (e.g., infections and acute rejection episodes) were linked to the most recent serum IgG levels. We obtained laboratory data from the computerized data warehouse, which contains all laboratory values obtained in the medical center. We included creatinine, complete blood count, plasma levels of calcineurin inhibitors, and IgG levels. Serum IgG levels were measured by nephelometry in the Special Laboratory of New York Presbyterian Hospital. Mild HGG was defined as a serum IgG level between 400 and 699 mg/dl. Severe HGG was defined as serum IgG level less than 400 mg/dl.

We routinely used daclizumab, tacrolimus, azathioprine (2 mg/kg), and corticosteroids after transplantation. Prednisone was tapered to 10 mg daily over 12 wk. Target trough tacrolimus levels were 10–15 ng/ml in the first 6 mo and 8–12 ng/ml thereafter. Tacrolimus was changed to cyclosporine A if drug toxicity occurred. Target cyclosporine A trough levels were 300 to 400 ng/ml in the first 6 mo and 250 to 350 ng/ml thereafter. Mycophenolate mofetil (MMF) was substituted for azathioprine when refractory or recurrent acute rejection occurred or when patients experienced drug toxicity.

Statistical Analysis

Continuous variables were summarized by the mean $\pm$ SD or median (interquartile range [IQR]). Categorical variables were summarized by the frequency and 95% confidence interval (CI). Correlations between

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Correspondence and requests for reprints should be addressed to Selim M. Arcasoy, M.D., Division of Pulmonary, Allergy, and Critical Care Medicine, 622 West 168th Street, PH 14, Room 104, New York, NY 10032. E-mail: sa2059@columbia.edu

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pretransplant IgG levels and other continuous variables were assessed using Pearson's correlation coefficients or Spearman's rho, as appropriate. Student's *t* tests and Wilcoxon rank sum tests were used to compare continuous variables of patients with pretransplant HGG with those with normal IgG levels. Dichotomous variables were compared using χ^2 tests or Fisher's exact tests. Paired comparisons of dichotomous variables before and after transplantation were performed using McNemar's tests.

We used multiple linear regression to assess predictors of pretransplant IgG level. We used purposeful selection of covariates to construct the model using variables that were significantly correlated on bivariate analyses and retained variables in the model with *p* values of 0.10 or less.

Linear mixed-effects models were used to assess predictors of post-transplant IgG level. Potential predictors and time from transplant were modeled as fixed effects, and patients were modeled using random effects. Logistic regression was used to assess predictors of post-transplant HGG; *p* values less than 0.05 were considered statistically significant. SAS version 9 (SAS Institute, Cary, NC) was used for all statistical analyses.

RESULTS

Forty consecutive patients were included in the cohort. The mean age was 49 ± 15 yr, and 23 patients (58%) were female. Thirty-four were non-Hispanic white, three were Hispanic white, two were non-Hispanic black, and one was Asian. Thirteen (33%) had COPD, 11 (28%) had cystic fibrosis or bronchiectasis, 13 (33%) had idiopathic interstitial pneumonia, one (3%) had pulmonary arterial hypertension, and two (5%) had sarcoidosis. None had a primary immunodeficiency-associated lung disease as an indication for lung transplantation. Twenty-four patients (60%) were using corticosteroids at the time of IgG level assessment before transplantation at a median dose of 10 mg (IQR, 0–20).

Pretransplantation IgG levels

The distribution of pretransplantation IgG levels is shown in Figure 1. Patients with COPD had significantly lower IgG levels at baseline than patients with other diagnoses (795 ± 278 mg/dl vs. $1,516 \pm 491$ mg/dl, $p < 0.0001$). IgG levels were inversely associated with patient age (Spearman's rho = -0.52 , $p = 0.0005$; Figure 2) and possibly with prednisone dose, although this was not statistically significant (Spearman's rho = -0.27 , $p = 0.09$). Multivariate analysis showed that a diagnosis of COPD was an independent predictor of lower IgG level (Table 1). In this analysis, increasing age and prednisone dose may have been

associated with lower IgG level, but this was not statistically significant. One interpretation of this analysis is that a patient with COPD on average had an IgG level that was 500 mg/dl lower than a patient with another indication for lung transplantation of the same age and using the same dose of prednisone. Subset analysis of only patients older than 50 yr at the time of IgG assessment ($n = 24$) showed that patients with COPD still had lower IgG levels than patients with other diagnoses (790 ± 290 mg/dl vs. $1,349 \pm 471$ mg/dl, $p = 0.002$); there was no association between age or corticosteroid dose in this subset analysis (data not shown).

Before lung transplantation, no patient had severe HGG (upper 97.5% bound, 8%), and the prevalence of mild HGG was 15% (95% CI, 6–30%). Patients with mild HGG were older ($p = 0.03$) and were more likely to have a diagnosis of COPD ($p = 0.005$) than patients with normal IgG levels (Table 2). There was no association between HGG and corticosteroid use.

Post-transplant IgG Levels

Thirty-six patients (90%) underwent bilateral sequential lung transplantation, and four (10%) underwent single lung transplantation. Thirty-six (90%) patients received tacrolimus, and seven (18%) received cyclosporine A. Thirty-eight (95%) received azathioprine, and six (15%) received MMF. Five (13%) were treated with sirolimus. The medication usage totals more than 100% because some patients received more than one therapy sequentially.

IgG levels were assessed a mean of seven times per patient after lung transplantation. The median follow-up time was 80 d (IQR, 45–147 d). There was no association between a diagnosis of mild or severe HGG and the number of IgG assessments performed ($p = 0.54$).

There was a significant reduction from baseline in IgG levels after lung transplantation (Figure 3). The median reduction in IgG levels from pre- to first post-transplant assessment was 442 mg/dl (IQR, 211–930 mg/dl; $p < 0.0001$). Patients taking MMF had significantly lower IgG levels compared with patients who were not (Table 3). Pretransplant IgG was also a strong predictor of posttransplant IgG level. Higher prednisone dose ($p = 0.12$) and longer duration from the time of transplantation ($p = 0.06$) may have been associated with lower IgG levels but were not statistically significant.

There were significant increases in mild and severe HGG after transplantation. Twenty-three patients (58%; 95% CI, 41–73%)

Figure 1. Histogram of pretransplant serum IgG levels. The normal range of serum IgG is 700–1,700 mg/dl.

Figure 2. Scatterplot of age versus pretransplant serum IgG level.

were diagnosed with mild HGG after transplantation ($p = 0.0002$ compared with the prevalence before transplantation). Six patients (15%; 95% CI, 6–30%) had severe HGG after transplantation ($p = 0.03$ compared with before transplantation). The median times from transplantation to diagnosis of mild and severe HGG were 33 d (range, 16–322 d) and 190 d (range, 30–458 d), respectively. Four of the six patients with severe post-transplant HGG had mild HGG before transplantation. The only significant predictor of post-transplant severe HGG was lower pretransplant IgG level ($p = 0.02$).

There were no significant associations between posttransplant IgG levels or post-transplant HGG and clinical symptoms (gastrointestinal, respiratory, fever), pneumonia, use of antibiotics, cytomegalovirus disease, detection of cytomegalovirus DNA by polymerase chain reaction, acute rejection, or abnormality in pulmonary function (data not shown).

DISCUSSION

We have shown that the prevalence of mild HGG is 15% at baseline in patients who eventually undergo lung transplantation. Pretransplant mild HGG affected only patients with COPD and was independent of corticosteroid use. The cumulative incidences of mild and severe HGG increased significantly after lung transplantation. Lower pretransplant IgG levels and the use of MMF predicted significantly lower post-transplant IgG levels. The only factor that predicted severe post-transplant HGG was pretransplant IgG level.

COPD was the major determinant of pretransplant (and therefore post-transplant) IgG levels and HGG. Although previous studies have attributed lower IgG levels in patients with COPD to smoking, the use of corticosteroids, or older age, our findings effectively refute these hypotheses (8–10). All patients in our study with pretransplant HGG were current nonsmokers, confirmed by serial testing of urine nicotine metabolites. In addition,

patterns of corticosteroid use and dosage were similar in patients with and without HGG. COPD remained significantly associated with pretransplant HGG after adjusting for the effects of age and prednisone dose, and subset analysis of patients older than 50 yr confirmed the association between COPD and lower IgG levels. This study and our previous study support an underlying biologic predisposition to lower IgG levels in patients with COPD even after removal of the diseased lungs. Whether HGG contributes to the risk of COPD or if COPD (perhaps through malnutrition or other factors) lowers IgG levels is unknown. Future studies of this association should clarify this.

The cumulative incidence of severe HGG after lung transplantation in the current study (15%) is virtually identical to the prevalence found in our previous study of an earlier cohort of patients from our center (14%) (6). These estimates are somewhat less than that of the only other study of HGG after lung transplantation but exceed those in cardiac transplant patients (11, 12). We have confirmed that severe HGG in patients with advanced lung disease (without a primary immunodeficiency state) is strictly a posttransplant phenomenon due to preexisting immunologic factors. However, mild HGG does exist before transplantation and occurs frequently in the early post-transplant period.

TABLE 1. PREDICTORS OF PRETRANSPLANT IgG LEVEL

	Coefficient	SE	p Value
Constant	2,017.1	243.7	0.001
COPD	−565.3	157.5	0.001
Prednisone dose	−6.1	3.4	0.08
Age	−9.5	5.1	0.07

Definition of abbreviation: COPD = chronic obstructive pulmonary disease.

TABLE 2. PRETRANSPLANT CHARACTERISTICS

	Mild HGG (<i>n</i> = 6)	Normal IgG (<i>n</i> = 34)	p Value
Age, yr	60 ± 3	47 ± 1	0.03
Sex, female	4 (67%)	19 (56%)	0.7
Ethnicity/race			1.0
Non-Hispanic white	6 (100%)	28 (82%)	
Hispanic white	0	3 (9%)	
Black	0	2 (6%)	
Asian	0	1 (3%)	
Diagnosis			0.005
Cystic fibrosis/bronchiectasis	0	11 (32%)	
COPD	6 (100%)	7 (21%)	
Idiopathic interstitial pneumonia	0	13 (38%)	
Pulmonary arterial hypertension	0	1 (3%)	
Sarcoidosis	0	2 (6%)	
Prednisone use	3 (50%)	21 (62%)	0.67
Prednisone dose, mg	5 (0–10)	10 (0–20)	0.3

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; HGG = hypogammaglobulinemia.

Figure 3. Pretransplant and linear model of post-transplant IgG levels with 95% confidence intervals.

The use of MMF was also associated with low IgG levels in our study. MMF has well known negative effects on antibody production through inhibition of T- and B-cell proliferation (13). A randomized clinical trial of MMF compared with azathioprine after kidney transplantation showed clear reductions in IgG levels caused by MMF. Our study confirms that MMF reduces IgG production in lung transplant recipients, independent of other factors, such as pretransplant IgG levels and patient diagnosis.

Although the use of MMF is associated with significantly lower post-transplant IgG levels in lung transplant recipients, this therapy does not account for the occurrence of mild or severe HGG in these patients. This apparent inconsistency may be easy to reconcile. Although most lung transplant recipients are characterized by normal IgG levels preoperatively, there is a subset of lung transplant candidates (commonly with COPD) who have lower IgG levels at baseline and may be particularly susceptible to the nonspecific effects of immunosuppressive medications, resulting in profoundly reduced levels. These 15% of patients are destined to develop severe HGG, with or without MMF and independent of corticosteroid dose, because they have a preexisting defect in antibody production. On the other hand, antibody production in lung transplant recipients with normal preoperative IgG levels is adversely affected by the use of MMF.

We and others have previously shown a significant association between severe HGG and adverse events after lung transplantation (6, 11, 14). In this study, however, we did not find a significant association between the absolute value of posttransplant IgG or posttransplant severe HGG with clinical outcomes such as infectious complications. This is likely attributable to a shorter and earlier follow-up period after transplantation in the current study and differences in patient cohorts.

Our study has several limitations. By nature, a cohort of lung transplant recipients is highly selected and may differ from other

patients with lung disease. However, pre- and post-transplant IgG measurement was performed routinely in consecutive transplant recipients, making these findings generalizable to other lung transplant populations. Our study was intended to examine the course and mechanisms of HGG early after lung transplantation; therefore, a relatively short follow-up period may explain the absence of a significant association between HGG and clinical outcomes. A study with a longer follow-up period would clarify the influence of HGG on clinical outcomes such as bronchiolitis obliterans and infections.

In summary, we have shown that patients with COPD have lower pretransplant IgG levels than others and have an increased risk of mild HGG. After lung transplantation, there is a significant drop in IgG levels and an increase in the incidences of mild and severe HGG. Pretransplant IgG level and the use of MMF determine posttransplant IgG levels, and pretransplant IgG level predicts an increased risk of post-transplant mild and severe HGG. The mechanistic link between COPD and HGG and the role of cytokine polymorphisms in the development of post-transplant HGG should be further investigated. The efficacy of post-transplant IgG supplementation remains to be proven in a randomized clinical trial with longer-term follow-up.

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TABLE 3. PREDICTORS OF POST-TRANSPLANT IgG LEVEL

	Coefficient	SE	p Value
Constant	548.8	98.4	< 0.0001
Use of mycophenolate mofetil	-86.0	41.6	0.04
Prednisone dose	-2.5	1.5	0.10
Pretransplantation IgG level	0.2	0.06	0.003
Time from transplantation	-0.2	0.1	0.09

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