

Serum Immunoglobulin G is a Marker for the Risk of Opportunistic Infection in Steroid-dependent Severe Asthmatic Patients

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Abstract

Background Approximately 10% of asthmatic patients are refractory to inhaled corticosteroids and therefore need long-term oral corticosteroid therapy, which is associated with a risk of opportunistic infections due to immunosuppression.

Objective To ascertain the applicability of serum Immunoglobulin G (IgG) as a marker for predicting the risk of opportunistic infections in patients undergoing oral corticosteroid therapy.

Methods Three thousand asthmatics were screened, and 14 patients who had been administered daily oral corticosteroids for more than two years were enrolled. The patients enrolled were maintained under observation with ordinary check-ups and treatments for one year. After the observation period, the patients were divided into two groups according to the presence (OPI) or absence (Non-OPI) of opportunistic infections during the period. The differences in the clinical parameters between the groups were investigated.

Results There were no statistically significant differences in age, forced expiratory volume in 1 second (FEV₁), smoking status or serum albumin between the groups. The serum IgG level of the OPI group was significantly lower than that of the Non-OPI group (567.2±151.1 mg/dL vs. 931.6±198.8 mg/dL, $p<0.01$). The average total dose of corticosteroids administered during the one year period was higher in the OPI group (2,633±554.2 mg) than that in the Non-OPI group (1,793±466.2 mg) ($p<0.05$). There was a significant correlation between the serum IgG and total dose of corticosteroids administered during the one-year period ($r=-0.75$, $p<0.01$). The area under the receiver operating characteristic curve regarding the serum IgG and incidence of opportunistic infections was 0.97, which suggests that the serum IgG level has a high accuracy for predicting the risk of opportunistic infections.

Conclusion The serum IgG was therefore found to be a useful marker for predicting the risk of opportunistic infections in steroid-dependent asthmatics.

Key words: opportunistic infection, corticosteroid, severe asthma, serum immunoglobulin G, biomarker

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Introduction

Asthma is a serious worldwide public health problem for all age groups, and is characterized by chronic airway inflammation. Corticosteroids are the most effective anti-inflammatory medication for bronchial asthma (1). Recently,

with the development of inhaled corticosteroids (ICS), the treatment of asthma has been dramatically improved. The introduction of ICS has markedly reduced hospitalizations (2), and deaths (3) from asthma. However, approximately 10% of asthma patients are ICS-refractory (4) and sometimes need long-term oral-corticosteroid therapy to reduce their airway inflammation and relieve symptoms. How-

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ever, long-term systemic corticosteroid therapy sometimes causes adverse effects, including opportunistic infections.

Opportunistic infections are caused by pathogens that do not normally infect healthy individuals with normal immune systems. These infections are seen in patients with immunodeficiency or immunosuppression, such as that induced by cancer chemotherapy, genetic predisposition, human immunodeficiency virus (HIV) or administration of immunosuppressive agents, and can cause serious health problems. Corticosteroids are one of the most well-known immunosuppressive agents (5); they inhibit various cytokines and chemokines that are important for the innate immune response, and abrogate the functions of immune cells, including lymphocytes, neutrophils and cells of the monocyte-macrophage lineage (6).

Regarding the association between the dose of corticosteroids administered and the risk of opportunistic infection, it has been reported that a daily dose of less than 10 mg (prednisolone equivalent milligrams), or a cumulative dose (prednisolone equivalent milligram $\times$ number of days administered systemic corticosteroids) of less than 700 mg, did not increase the risk of infection (7). However, the authors of that study also reported that the risk of infection was related to the type of underlying disease: the risk was high in patients with neurological diseases but low in those with intestinal diseases (7). For this reason, it is crucial to establish a cut-off level for steroids, above which there is an increased risk of opportunistic infection in steroid-dependent asthma. Furthermore, it would be highly desirable if the risk of opportunistic infection could be predicted by suitable biomarkers.

In this study, we focused on steroid-dependent asthmatic patients, and investigated the relationship between opportunistic infections and clinical parameters. The aim of this study was to ascertain the applicability of using serum Immunoglobulin G (IgG) as a marker for predicting the risk of opportunistic infections in patients undergoing long-term oral corticosteroid therapy.

Materials and Methods

Study design

This was a case-control study involving exclusively steroid-dependent asthmatic patients attending the Department of Allergy and Respiratory Medicine, The Fraternity Memorial Hospital, one of the leading teaching hospitals in respiratory medicine in Japan, from 2008 to 2009. Three thousand asthmatic patients who visited our hospital were included in the screening, and 14 patients who had been administered daily oral corticosteroids to control their asthma symptoms for more than two years were enrolled in this study. The diagnosis of asthma was based on the Global Initiative for Asthma (GINA) guidelines (8). Current smokers, patients with chronic obstructive pulmonary disease (COPD), patients with malignant diseases and patients with

other immune diseases (for example, rheumatoid arthritis) were excluded from the study.

The enrolled patients were maintained under observation with ordinary medical check-ups and treatment for one year, during which spirometry and blood tests (including serum albumin and serum IgG measurements) were carried out when patients were at baseline conditions without acute exacerbations of asthma or infections. At the end of the one year period, the patients were divided into two groups according to the presence or absence of opportunistic infections during the observation period, and differences in the clinical parameters between the groups were investigated. The dose of corticosteroids administered was calculated based on equivalents to prednisolone according to published data on the relative potencies of hormonal effects (9). The study was approved by the Institutional Review Board of the Fraternity Memorial Hospital and patient anonymity was preserved at every stage of the study.

Statistical analyses

The data are presented as the means $\pm$ standard deviation (SD). Comparisons between groups were performed using the Mann-Whitney rank sum test for groups that are not normally distributed. For the correlation analysis between the groups, the Spearman rank correlation coefficient was used. All statistical analyses were performed using the Graph Pad Prism 4 Software program (GraphPad Software Inc, San Diego, CA).

Results

Clinical characteristics of subjects

Fourteen of 3,000 asthmatic patients visiting to our hospital had been administered a daily dose of oral corticosteroids for more than two years at the time of the enrollment. Five of these 14 patients suffered from opportunistic infections during the observation period.

The infections suffered were herpes zoster (n=1), local candida infection (n=1), methicillin-resistant *Staphylococcus aureus* (MRSA) pneumonia (n=1), aspergillosis (n=1) and a fungal infection (n=1). The patient who had herpes zoster had no other underlying diseases, such as malignancy, and was successfully treated with acyclovir. The patient with a local candida infection showed a high beta-D-glucan level (>300 pg/mL) and was positive for the candida antigen, and was successfully treated with voriconazole. The patient with MRSA pneumonia showed infiltration of the right lower lung field and positive testing results for MRSA in the sputum, and successfully treated with teicoplanin. The patients who had aspergillosis or a fungal infection with unknown foci were positive for the candida antigen and had a high level of beta-D-glucan, respectively, and both patients were treated successfully with antifungal agents.

The mean age of the patients with opportunistic infections (OPI group) was 52.8 ± 18.6 , and that of the patients without

Table. Patient Characteristics

	Non-OPI	OPI	p
N	9	5	
Age (yr)	72.8±9.78	52.8±18.6	NS
Sex (F/M)	3/6	0/5	
Pack-years	0±0	10±23	NS
FEV ₁ (%predicted)	41.0±8.8	75.4±32.2	NS
FEV ₁ /FVC (%)	55.0±12.4	72.4±11.6	NS
Serum albumin	4.1±0.5	4.2±0.8	NS

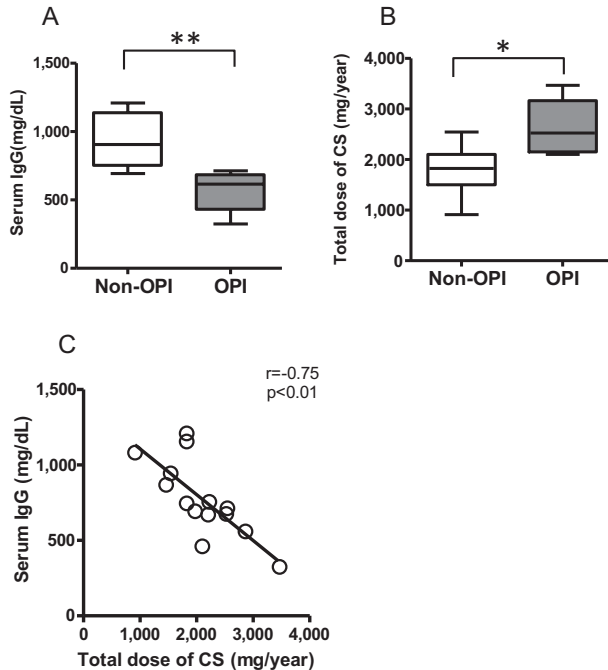


Figure 1. The serum IgG level is lower in the OPI group than that in the Non-OPI group, and correlates with the total dose of corticosteroid (CS) administered per year. Steroid-dependent severe asthmatic patients were divided into two groups according to whether they had episodes of opportunistic infection: the OPI group suffered from opportunistic infections during the observation period, while the Non-OPI group did not. The serum IgG levels (Panel A) and total dose of CS administered per year (Panel B) were compared between the groups. A correlation analysis was performed between the serum IgG levels and total dose of CS administered per year (Panel C). *: $p<0.05$, **: $p<0.01$

opportunistic infections (Non-OPI group) was 72.8 ± 9.78 (Table). The mean age was lower in the OPI group compared to that in the Non-OPI group, but there was no significant difference in age between the groups. The mean FEV₁ in the Non-OPI group ($41.0\pm8.8\%$) was lower than that in the OPI group ($75.4\pm32.2\%$), but there was also no significant difference in the FEV₁ between the groups. The smoking status (pack-years, 0 ± 0 in the Non-OPI group, 10 ± 23 in the OPI group) and serum albumin level (4.2 ± 0.8 g/dL in the Non-OPI group, 4.1 ± 0.5 in the OPI group), a marker of the nutritional state, showed no significant differences between the groups (Table). The medications administered to

the patients were inhaled corticosteroids (14 patients), long-acting muscarinic antagonists (3 patients), long-acting β_2 -agonists (14 patients) and theophylline (14 patients).

Serum IgG as a marker for risk of opportunistic infection

The mean serum IgG level of the OPI group was significantly lower than that of the Non-OPI group (567.2 ± 151.1 mg/dL vs. 931.6 ± 198.8 mg/dL, $p<0.01$) (Fig. 1A). The total dose of corticosteroids administered during the one year period (including on-demand use) in the OPI group ($2,633\pm554.2$ mg) was higher than that in the Non-OPI group ($1,793\pm466.2$ mg) ($p<0.05$) (Fig. 1B). In addition, there was a significant correlation between the serum IgG level and total dose of corticosteroids administered in the one year period in both groups ($r=-0.75$, $p<0.01$) (Fig. 1C).

Receiver operating characteristic (ROC) curve analysis using IgG as a biomarker

ROC analysis of the association between the serum IgG level and opportunistic infections (Fig. 2A) revealed that the area under the curve (AUC) was 0.97 ($p<0.01$), which suggests a high level of accuracy. The cut-off level for IgG in the presence or absence of an opportunistic infection was 729 mg/dL, with a specificity of 100% and sensitivity of 88%. The AUC of the ROC curves of the total dose of corticosteroids administered during one year and the incidence of opportunistic infections was 0.89 ($p=0.02$) (Fig. 2B). The most reliable cut-off dose-level was calculated to be 2,375 mg/year (equivalent to 6.5 mg/day), with a specificity of 88.9% and a sensitivity of 60.0%.

We also evaluated the repeatability of using the serum IgG as a biomarker by a method proposed by Bland and Altman (10). Nine out of the 14 patients had undergone repeated serum IgG measurements for a more than three month interval under the same clinical conditions. The standard deviation of the differences in the two measurements was 45.7. All values (differences between two measurements) were within 2SD, suggesting that the marker had good repeatability.

Discussion

It would be highly desirable to be able to predict the risk of opportunistic infection in patients receiving long-term oral corticosteroids. In this study, we have identified two useful parameters for predicting the risk of opportunistic infections. The first is the serum levels of a biomarker, IgG. The statistical analysis indicated that both the sensitivity and specificity of using the serum IgG level as a predictor of an opportunistic infection are high. Furthermore, the AUC of the ROC curve for IgG suggests that its accuracy as a biomarker is high (Fig. 2A). In addition, using the serum IgG level as a biomarker showed good repeatability when it was assessed by a method proposed by Bland and Altman (10). By monitoring the serum IgG, clinicians can eas-

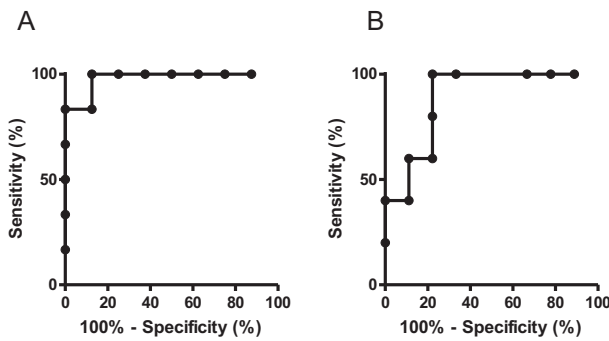


Figure 2. Results of the ROC analysis of the serum IgG level and total dose of CS administered per year as markers to predict the risk of developing an opportunistic infection in steroid-dependent severe asthmatics. Serum IgG (Panel A), total dose of CS administered per year (Panel B)

ily obtain information about the risk of opportunistic infections in patients treated with daily oral corticosteroids. The second parameter is the cut-off dose-level of corticosteroid, which we have ascertained, above which there is a statistically significant risk of opportunistic infection in steroid-dependent severe asthmatic patients.

Asthma is an allergic inflammatory disease, mainly caused by mast cells and Th2-immune system abnormalities (11). In asthmatic patients, elevation of the IgE levels is sometimes detected. However IgG production is not normally affected. Therefore, the IgG level is an independent marker for the immunosuppression caused by corticosteroid therapy in asthmatic patients.

Immunoglobulins play an important role in the immune system and are produced by plasma cells. The serum IgG level is elevated during monoclonal and/or polyclonal expansion of plasma cells. Serum IgG is decreased by the loss of, and/or reduced production of, immunoglobulins. The main causes of the loss of immunoglobulins are nephrotic syndrome, burn injury and protein losing enteropathy, and the main cause of a decrease in immunoglobulin production is immunodeficiency, which may in turn be caused by a variety of factors. Corticosteroids are among the best known agents that suppress the proliferative response of B cells (12) and, as a result of this suppression, the serum IgG levels are observed to decrease.

Corticosteroids inhibit a wide range of immune functions; the effect is not limited to IgG production (6). However, monitoring the state of the immune system using many parameters is difficult and costly. The serum IgG level is evaluated during routine laboratory testing in hospitals in many countries. The simplicity of measurement of IgG offers great advantages for using it as a biomarker. In addition, our data demonstrated that the IgG level may be a surrogate marker of the immunosuppressive state, even though it represents solely the B cell function.

Cell-mediated adaptive immune responses, such as the T-cell response, mainly contribute to host defense against opportunistic pathogens such as *Pneumocystis jirovecii* pneu-

monia (13), fungal infections (14), (15) and tuberculosis (16). Therefore, it might be better to monitor T cell immunity to predict the risk of these infections. However, monitoring T cell immunity is costly. Furthermore it is time-consuming and is not available as a routine laboratory test in hospitals in many countries, except in HIV-prevalent regions. For these reasons, and together with the fact that corticosteroids suppress both T- and B-cell immunity, monitoring the serum IgG level is far simpler, more cost effective, and potentially better as a surrogate marker for assessing the risk of opportunistic infections in the clinical setting.

Corticosteroids are strong anti-inflammatory agents. The introduction of steroid therapy has led to improvements in the prognosis of various chronic inflammatory diseases, including rheumatoid arthritis, systemic lupus erythematosus, sarcoidosis and asthma. Therefore, optimum use of corticosteroids brings huge benefit to patients after considering the balance between the risks and benefits. If doctors reduce the dose of oral corticosteroids too quickly, for instance because of an unnecessary fear of adverse effects, this might lead to exacerbation of the inflammatory disease. Setting a cut-off level according to the estimated risk of opportunistic infection will help to ensure effective therapeutic planning for corticosteroid therapy.

The major limitation of the study is that we could not perform a multiple regression analysis to select independent markers due to the small study population. However, the serum IgG of the subjects in this study showed no significant correlations with the age or serum albumin level of the patients, although, in general, both the serum albumin and IgG levels are age-dependent.

In conclusion, the serum IgG level could be a useful marker to predict the risk of opportunistic infections. The serum levels of IgG need to be monitored in steroid-dependent severe asthmatic patents. When the serum IgG levels dip lower than the cut-off levels, monitoring patients for markers of opportunistic infections, such as beta-D-glucan, should be carried out to detect early-stage infection and allow for prompt treatment. At the same time, the serum IgG levels could represent a useful parameter to examine empirical therapies for preventing opportunistic infections.

The authors state that they have no Conflict of Interest (COI).

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