

Response to inhaled corticosteroids on serum CD28, quality of life, and peak expiratory flow rate in bronchial asthma

Anubhuti Gupta, M.B.B.S.,¹ Lalit K. Gupta, M.D.,¹ Harmeet S. Rehan, M.D.,¹ and Anupam Prakash, M.D.²

ABSTRACT

Background: CD28 is a 44 kDa glycoprotein that is important in initiating T-cell responses and that results in increased T-cell proliferation and interleukin-2 production. This study estimated the serum CD28 levels in patients with asthma and evaluated the effect of inhaled corticosteroids (ICS) on the levels of CD28, the peak expiratory flow rate (PEFR), and quality of life (QOL).

Methods: This prospective, open-label, observational study enrolled 40 adult patients with asthma of mild-to-moderate severity who were started on ICS and 40 healthy controls. Patients with bronchial asthma were evaluated for their serum CD28 level and QOL by using Mini Asthma Quality of Life Questionnaire scores, severity of symptoms, and PEFR at baseline and after 4 weeks.

Results: The mean (standard deviation [SD]) serum CD28 concentration in patients with asthma was 107 ± 4.98 ng/mL, which was significantly elevated ($p < 0.01$) compared with the healthy individuals (37.67 ± 18.28 ng/mL). ICS treatment for 4 weeks reduced the mean (SD) serum CD28 levels to 40.9 ± 2.82 ng/mL. PEFR increased significantly, from 190.75 ± 10.55 to 263 ± 10.69 L/min ($p < 0.01$) after 4 weeks. Similarly, the mean (SD) Mini Asthma Quality of Life Questionnaire scores significantly increased, from 36.90 ± 10.31 on day 0 to 70.63 ± 11.56 on day 28 after ICS therapy ($p < 0.01$). A negative correlation between the elevations of serum CD28 levels was seen with QOL.

Conclusion: The serum CD28 concentration, a marker of inflammation, is increased in bronchial asthma and reduced by ICS therapy. ICS also improved QOL scores and objective clinical outcomes in patients with asthma.

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Bronchial asthma is a complex and heterogeneous disease characterized by spontaneous airflow limitation, lung tissue remodeling, increased serum immunoglobulin E concentration, and airway hyperresponsiveness, with the infiltration of lymphocytes and eosinophil into the airway submucosa. It is characterized by several phenotypic and endotypic variants with different pathophysiologic mechanisms and involvement of various cytokines. Parameters considered when defining asthma endotypic variants are clinical characteristics, biomarkers, lung functioning, genetics, histopathology, epidemiology, and response to treatment. The various endotypes of asthma are the following: aspirin sensitive, allergic bronchopulmonary mycosis, allergic asthma (adults), preschoolers with wheezing and positive asthma predictive

indices, severe late-onset hypereosinophilic asthma, and asthma in cross-country skiers.^{1,2}

The average annual global asthma prevalence in adults is 7.7%, and the prevalence is increasing with each passing year.³ Currently, 4.3% of the population worldwide has asthma. Individuals with asthma face deterioration in their quality of life (QOL), which places a financial and psychological burden on their family and society at large.⁴ CD28 is a 44 kDa glycoprotein that is important in initiating T-cell responses and results in an increased T-cell proliferation and interleukin 2 production. CD28 can be produced either by shedding of the membrane form or by alternative messenger RNA splicing.^{5,6} Previous research established the role of CD28 in various allergic conditions, where it acts as a costimulatory molecule in the T-cell activation, but its importance specifically in asthma is unexplored. More specific therapies may be developed by using CD28 as an appropriate target in the management of bronchial asthma.^{7–9}

Despite the complexity of asthma pathogenesis, confidence in the fundamental role of T-helper 2 (Th2) cells in asthma pathobiology has led pharmaceutical and biotechnology companies to develop new asthma treatments that target Th2 cytokines or their receptors. T cells are activated by binding of the T-cell receptor to major histocompatibility complex–

From the ¹Department of Pharmacology, Lady Hardinge Medical College and Shrimati Sucheta Kriplani Hospital, New Delhi, India, and ²Department of Medicine, Lady Hardinge Medical College and Shrimati Sucheta Kriplani Hospital, New Delhi, India. No external funding sources reported.

The authors have no conflicts of interest to declare pertaining to this article. Address correspondence to Lalit K. Gupta, M.D., Department of Pharmacology, Lady Hardinge Medical College and Shrimati Sucheta Kriplani Hospital, New Delhi 11001, India.

E-mail address: lkg71@rediffmail.com

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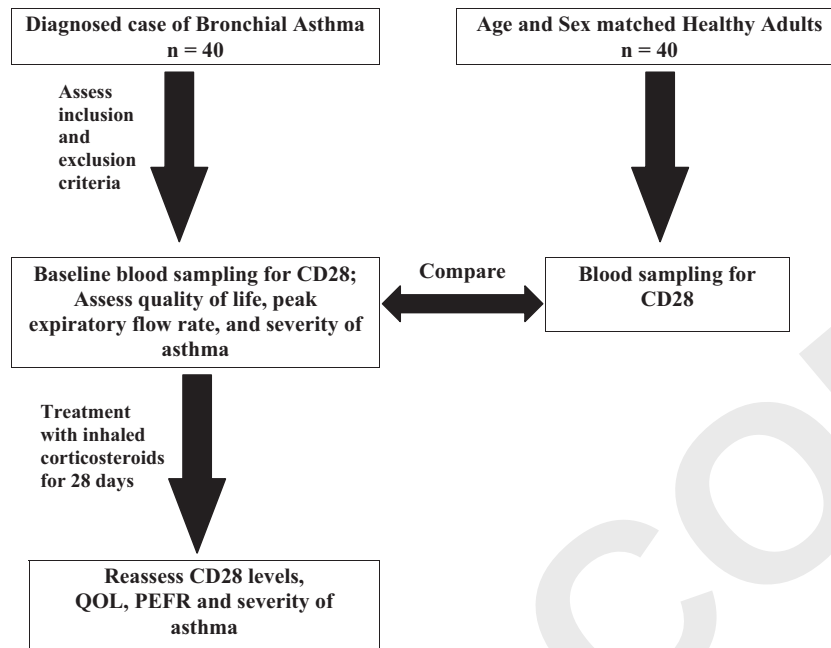


Figure 1. Study methodology flowchart.

antigens presented by antigen presenting cell and by a second, nonspecific, costimulatory signal provided by binding of B7 molecules (B7-1 and B7-2) to CD28/cytotoxic T-lymphocyte associated antigen-4. When both signals are provided to the T cells, they proliferate and hence secrete cytokines. In the absence of a costimulatory signal, T cells that bind to the antigen die or become anergic. Also, T-cell survival is enhanced by CD28 costimulation.^{10,11}

Gogishvili *et al.*¹² and Mathur *et al.*⁸ studied animal models to evaluate the importance of costimulation in bronchial asthma. CD28 critically regulates the allergic Th2 phenotype. These results emphasize the importance of the potential for CD28 blockade in the treatment of atopic airway disease.^{8,12} Autoantibodies against CD28 could be a novel mechanism in allergic inflammation.¹³ There are studies that indicated that allergic airway inflammation requires CD28 and cytotoxic T-lymphocyte associated antigen-4 receptors as important regulatory components. Because CD28 acts at the first level of inflammatory cascade, *i.e.*, costimulation of antigen presenting cell with both Th1 and Th2, it would prove useful to block CD28 to treat most of the asthma endotypes. Preclinical data are available in justifying further studies on the role of CD28 in asthma treatment. *In vitro* responses of allergen- and antigen-specific T cells of patients with allergy and control persons without allergy are equally dependent on costimulation *via* the CD28-CD86 pathway, which indicates that inhibition of this pathway may prevent complete activation of allergen-specific T cells in individuals with allergy *in vivo*.^{2,14,15} This study estimated the serum CD28 levels in patients with asthma and compared with

healthy controls and also evaluated the effect of inhaled corticosteroids (ICS) on the levels of CD28, QOL, and the peak expiratory flow rate (PEFR).

METHODS

This prospective, open-label, observational study was conducted in the Department of Pharmacology and Medicine, Lady Hardinge Medical College, between December 2014 and March 2016 after approval from the institutional ethics committee for human research. Patients diagnosed with asthma of mild-to-moderate severity (age group range, 18–55 years for either sex) who were started on ICS according to guidelines by the Global Initiative for Asthma^{16–18} who presented to the medicine unit of Smt. Sucheta Kriplani Hospital, New Delhi, were included. Pregnant women and active smokers were excluded. Patients with severe asthma or any other coexistent active pulmonary infection or who had taken corticosteroids in any form in the past 4 weeks were also excluded. The study was registered with Clinical Trials Registry—India (CTRI/2015/07/005977).

The study methodology is summarized in Fig. 1. In this pilot study, a convenient sample size of 40 patients and 40 healthy controls was taken. Written informed consent was taken from all the participants, and anti-asthma medication was prescribed according to the Global Initiative for Asthma guidelines. Before initiation of ICS, the baseline serum level of CD28 was measured in blood samples (3–5 mL) from each patient. The asthma severity score, *i.e.*, the severity of asthma score,¹⁹ QOL by QOLtec Mini Asthma Quality

Table 1 Demographic data of enrolled individuals

Parameter	Patients with Bronchial Asthma (n = 40)	Healthy Volunteers (n = 40)
Sex, no. (%)		
Men	10 (25)	10 (25)
Women	30 (75)	30 (75)
Age, mean $\pm$ SE, y	39.35 $\pm$ 1.969	38.65 $\pm$ 1.883

of Life Questionnaire (MiniAQLQ) and PEFr were assessed for each patient.^{20–22} All the patients were also prescribed an inhalational bronchodilator as and when needed.

At the time of follow-up, serum sampling, *i.e.*, 4 weeks after starting ICS, the patients were evaluated again for their QOL, severity of symptoms, and PEFr. PEFr is an easy-to-measure, clinical parameter that gives insight into the status of lung function of subjects with bronchial asthma. The 15-item MiniAQLQ is self-administered, and the patients were asked to recall their experiences during the previous 2 weeks and respond to each question on a seven-point scale. MiniAQLQ is reliable, responsive, valid, short, and easy to complete. It contains questions that pertain to the following four domains: symptoms, activity limitation, emotional function, and environmental stimuli.^{23,24} Venous blood samples taken from the participants (3–5 mL) were centrifuged at 5000 rpm for 10 minutes and serum separated within 2 hours of collection of the sample. Serum samples were stored at -20°C , until the estimation of the marker CD28 by using a commercial enzyme-linked immunosorbent assay kit (Human Soluble Cluster of Differentiation 28 ELISA kit; Sincere Biotech, Beijing, China). The aliquots were labeled with the name and patient identification code.

Statistical Analysis

Data were transformed, coded and entered into an MS-Excel spreadsheet version 14.3.9 (Microsoft Corp, Redmond, WA). The results are expressed as mean (standard deviation). The analysis of difference in CD28 levels in healthy subjects versus patients with asthma was done by using the two-tailed unpaired Student's *t*-test. Analysis of any significant change in the serum values of the marker was compared by using the two-tailed Student's *t*-test (paired). The correlation between the levels of CD28 and scores of asthma severity and QOL were calculated by using the Karl Pearson coefficient of correlation (the *r* value). Values with $p < 0.05$ are considered statistically significant.

RESULTS

Demographic data of individuals enrolled in our study are shown in Table 1. Of the subjects, 67.5% had

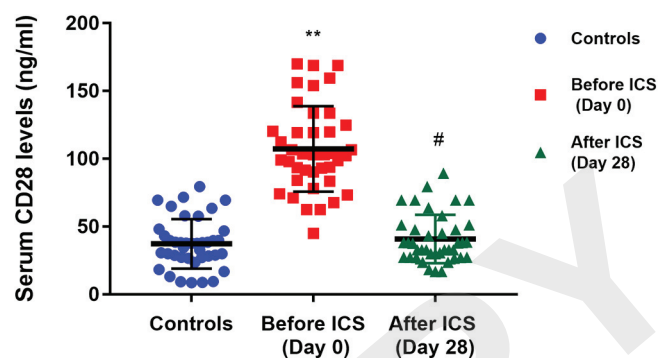


Figure 2. Serum CD28 levels in healthy subjects (controls) ($n = 40$) and subjects with bronchial asthma with exacerbation ($n = 40$). $**p < 0.01$, significant in comparison with the controls. CD28 in subjects with bronchial asthma before and after inhaled corticosteroid (ICS) ($n = 40$). Values are mean (standard deviation) compared by using the two-tailed unpaired *t*-test. $\#p < 0.001$.

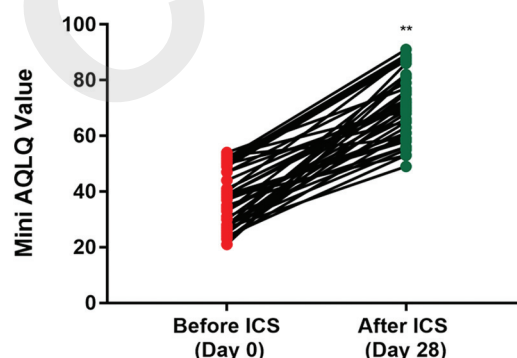


Figure 3. Comparison of the Mini Asthma Quality of Life Questionnaire (MiniAQLQ) before and after inhaled corticosteroid (ICS) by using the two-tailed paired *t*-test. Values are represented as mean (standard deviation). The 15-item, seven-point standardized self-administered MiniAQLQ. $**p < 0.001$.

mild asthma and 32.5% had moderate asthma, and none had severe asthma per the severity of asthma score. Twenty-two patients (55%) belonged to the age group of 24–44 years and 14 (35%) belonged to the age group of 45–60 years. None of the patients had a history of smoking or alcohol addiction. Twenty-seven patients (67.5%) had no family history of allergic disorder or asthma in the first-degree relatives. Fewer than half of the total patients were employed in any kind of job (45%). The rest of the patients were either homemakers or unemployed. The serum CD28 concentration in patients with asthma was significantly elevated compared with healthy individuals; however, ICS treatment for 4 weeks reduced it significantly (Fig. 2). Similarly, QOL and PEFr showed significant improvement with ICS therapy (Figs. 3 and 4; Table 2). The design of the MiniAQLQ was such that the QOL scoring was inversely related to the actual clinical benefits of asthma. In our study, a decrease in MiniAQLQ

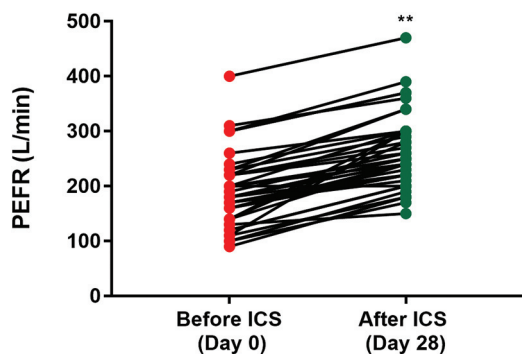


Figure 4. Comparison of the peak expiratory flow rate (PEFR) in subjects with mild-moderate bronchial asthma when experiencing an exacerbation ($n = 40$) and treated with inhaled corticosteroid (ICS) for the same; a comparison was made by using the two-tailed paired t -test. $**p < 0.001$.

Table 2. Mean serum CD28 levels, MiniAQLQ score, PEFR of patients before (day 0) and after inhaled corticosteroid therapy (day 28), $n = 40$

Parameter	At First Visit (day 0)	After 28 days
CD28, ng/mL	107 ± 4.98	$40.9 \pm 2.82^{**}$
MiniAQLQ score	36.90 ± 10.31	$70.63 \pm 11.56^{**}$
PEFR, L/min	190.75 ± 10.55	$263 \pm 10.69^{**}$

MiniAQLQ = Mini Asthma Quality of Life Questionnaire score; PEFR = peak expiratory flow rate.

$**p < 0.01$.

score was observed. A negative correlation between the elevations of serum CD28 was seen with patients' QOL (Fig. 5).

DISCUSSION

CD28 is important in the initiation of the inflammatory cascade in the airways.²⁵ The present study outlined that serum levels of CD28 are increased in patients with asthma. Neuber *et al.*²⁶ analyzed serum samples of healthy controls and those with bronchial asthma, and found that serum CD28 was significantly increased in subjects with asthma. This finding was similar to our present study. There are many monoclonal antibodies, such as mepolizumab,²⁷ reslizumab,²⁸ and benralizumab (against interleukin 5), which are recently being tried against various target markers in the inflammatory cascade. The high risk-to-benefit ratio of prescribing monoclonal antibodies to all the subjects with asthma clearly demonstrated the need for additional biomarker(s) to identify patients who would benefit from the monoclonal antibody.^{29–33} The results should provide new postulates for potential immunopathologic roles of costimulatory molecule CD28 in the exacerbation of allergic inflammation and the sugges-

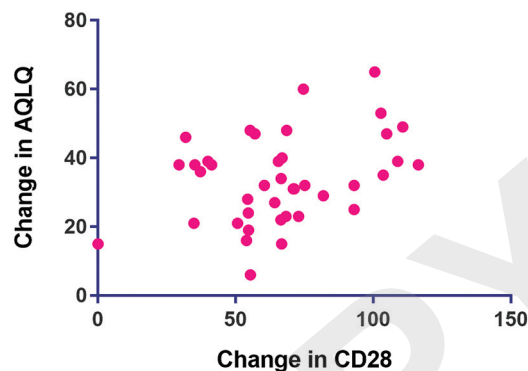


Figure 5. Correlation between the change in Mini Asthma Quality of Life Questionnaire (MiniAQLQ) scores and the change in serum CD28 levels by using the Karl Pearson coefficient of correlation ($r = 0.362$) ($p < 0.05$).

tion of new potential surrogate markers of allergic asthma.³⁴ CD28 may be considered a nonspecific inflammatory marker because it is also increased in other inflammatory conditions, such as atopic dermatitis, allergic rhinitis, and in demyelinating disorders, such as neuromyelitis optica and multiple sclerosis.^{27,35–38}

Another important finding in our study was that QOL improved significantly with ICS treatment for 4 weeks in patients with chronic asthma. Bhargava *et al.*³⁹ showed that QOL improves significantly after oral corticosteroid use in patients with an acute attack of asthma. The MiniAQLQ, an evaluative instrument that is sensitive to small within-subject changes over time and, therefore, appropriate for capturing the effect of ICS on the QOL of subjects with asthma was used in our study. Bronchial asthma is a chronic debilitating illness, which can be controlled but cannot be cured. Hence, it is important that an objective scale, such as the MiniAQLQ, is used to evaluate the impact of treatment on QOL. MiniAQLQ is a seven-point scale response-option questionnaire in which a mean overall change in the QOL score of 2.29 signifies a large improvement in QOL. A patient may feel and function better, but this may not be captured by one of the conventional clinical outcomes, *i.e.*, expiratory flow rates, symptoms, the need for medication, and airway responsiveness. The assessment of disease-specific QOL in clinical trials has gained importance recently because of an increased awareness of the importance of this aspect of treatment evaluation.⁴⁰

Also, another noteworthy observation in the present study was that the PEFR improved significantly after treatment with ICS for 4 weeks; however, improvement in the PEFR failed to show a significant positive correlation with the MiniAQLQ score, which could be because perception of better QOL comes earlier than the objective markers of clinical improvement. As the asthmatic exacerbation symptoms subside, there is a perception of improvement in QOL; whereas airway

inflammation and the biochemical and clinical markers would take months to come back to normal. The Global Initiative for Asthma guidelines¹⁸, also described that ICS significantly improve symptoms, inflammation, and airway function, and are regarded as one of the most-effective anti-inflammatory therapy for asthma.^{41,42} Previous studies identified the regulatory role of costimulatory molecule CD28 for inflammatory cell recruitment and Th2 cell differentiation after allergen provocation. Further elucidation of the roles and functions of costimulatory molecules requires the investigation of samples obtained from or near the site of inflammation, *e.g.*, induced sputum, bronchoalveolar lavage fluid, or exhaled breath condensate.^{43,44} In view of recent advances in the exploration of the therapeutic targeting of costimulation for the T-cell activation in allergic asthma, therapeutic agents, such as an antibody against the soluble form of costimulatory molecules and inducible costimulator, might be potential drugs for treating allergic asthma. CD28 is a prospective asthma treatment biomarker, which can be further refined by using pharmacogenomics.⁴⁵

CONCLUSION

This study showed that serum CD28 concentration, a marker of inflammation, was increased in allergic asthma and was reduced by ICS therapy. ICS also improved objective clinical outcomes and QOL scores in patients with asthma. Help from the identification of biomarkers can guide in choosing the best treatment, moving from a generalist approach to a more “tailored” therapy, for asthma.

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