



Circulating angiopoietin-1 and -2 in patients with stable and exacerbated asthma

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ABSTRACT

Background: Angiopoietin (Ang)-1 and -2 are involved in the pathogenesis of asthma and have been identified as markers of asthma severity.

Objective: To determine the relation between circulating angiopoietins and clinical variables of patients with asthma.

Methods: Fifty patients with bronchial asthma and 25 healthy controls were enrolled. Ang1 and Ang2 plasma levels were analyzed in patients with stable and exacerbated asthma.

Results: Plasma Ang1 levels were 28.4 ± 4.01 pg/mg in patients with bronchial asthma and 21.2 ± 5.21 pg/mg in healthy controls. Plasma Ang2 levels were 23.96 ± 1.38 pg/mg in patients with bronchial asthma compared with 36.8 ± 4.46 pg/mg in healthy controls ($P = .010$). The ratio of Ang2 to Ang1 was lower in patients with asthma than in control subjects. Plasma Ang1 concentrations were correlated with the ratio of forced expiratory volume in 1 second (FEV₁) to forced vital capacity (FVC), and plasma Ang2 levels were correlated with FEV₁ percentage of predicted, FEV₁/FVC, and total immunoglobulin E values. The ratio of Ang2 to Ang1 was correlated with FEV₁ percentage of predicted and FEV₁/FVC. Although plasma Ang1 levels tended to be lower in the exacerbated state than in the stable state in patients with asthma, Ang2 levels were higher in the exacerbated state than in the stable state in patients with asthma ($P = .001$). Plasma Ang2 levels were correlated with initial eosinophil proportions and initial neutrophil proportions. Plasma Ang2 levels and the ratio of Ang2 to Ang1 were correlated with blood eosinophil proportions in the exacerbated state.

Conclusion: These results indicate that circulating angiopoietins could be a useful marker of asthma exacerbation.

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Introduction

Asthma is characterized by chronic airway inflammation that is associated with structural changes, termed *airway remodeling*.¹ Increased angiogenesis is a well-documented feature of airway remodeling in asthma.¹ Angiogenesis can be initiated by endogenous angiogenic factors released from mesenchymal cells and/or inflammatory cells.²

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Among these factors, angiopoietins regulate vascular homeostasis by the endothelial tyrosine kinase Tie receptor.² Angiopoietin-1 (Ang1) supports endothelial stabilization by Tie2 activation,^{3–6} whereas angiopoietin-2 (Ang2) functions as a context-dependent Tie2 agonist and antagonist promoting pathologic angiogenesis, vascular permeability, and inflammation.^{3,7,8} Ang2 levels, normally constrained during homeostasis, are increased in endothelial cells during vessel remodeling, particularly in tumor vasculature, and in diseases associated with increased vascular permeability and endothelial dysfunction, such as sepsis, acute lung injury, lung cancer, and chronic obstructive lung disease.^{9–11} In addition to an increase in the Ang2/Ang1 ratio, decreased Tie2 levels have been reported in sepsis.^{12–15} Serum Ang1 levels also have been reported to be higher in patients with asthma than in healthy subjects, and the serum Ang1/Ang2 ratio has been correlated with lung function, suggesting that angiopoietins might be useful markers for the diagnosis of

asthma.¹⁶ Therefore, this study analyzed circulating levels of Ang1 and Ang2 in healthy subjects and patients with asthma and examined changes in these levels in patients with stable vs exacerbated asthma as indicated by changes in lung function parameters. This report is based on a prior publication¹⁷ in which the authors examined different measurements. In addition, the authors evaluated the possibility of a disease exacerbation marker for use in asthma monitoring.

Methods

Subjects

Clinical data were collected from 50 patients with asthma who were registered in an asthma cohort of the Genome Research Center for Allergy and Respiratory Diseases in Korea. The biospecimens and data used for this study were provided by the biobank of Soonchunhyang University Bucheon Hospital (Bucheon, Korea), a member of the Korea Biobank Network. Asthma diagnoses were based on Global Initiative for Asthma (GINA) guidelines.¹⁸ This study used the same sample as that examined by Moon et al,¹⁷ although several different measurements and analyses are presented in the present report. All subjects had a clinical diagnosis of asthma that was supported by at least 1 of the following criteria: (1) variability in maximum diurnal peak expiratory flow greater than 20% over the course of 14 days; (2) an increase in the patient's forced expiratory volume in 1 second (FEV₁) greater than 15% after inhalation of 200 to 400 μ g of albuterol; or (3) a positive methacholine provocation concentration less than 10 mg/mL that caused a decrease in FEV₁ of 20%. All subjects underwent standardized assessments, which included analyses of complete blood cell count with differential counts, immunoglobulin E (IgE) measurement, chest posteroanterior radiography, allergy skin prick tests, and spirometry. All data were collected at the time of diagnosis, before the administration of asthma medication, and during the period of exacerbation. Patients with asthma from the hospital cohort also were matched to healthy subjects according to age, sex, and body mass index. These healthy control subjects were recruited from among the spouses of patients or members of the general population and were included based on the following criteria: (1) negative responses on a screening questionnaire for respiratory symptoms and other allergic diseases; (2) FEV₁ values greater than 80% of predicted values; (3) provocation methacholine concentration less than 10 mg/mL that caused a decrease in FEV₁ of 20%; and (4) normal findings on simple chest radiographs. Of subjects who had been regularly followed for at least 2 years, 50 patients were diagnosed based on GINA guidelines.¹⁸ Asthma exacerbation was defined according to GINA guidelines as episodes of a progressive

increase in shortness of breath, cough, wheezing, or chest tightness, or some combination of these symptoms, accompanied by decreases in expiratory airflow and use of systemic corticosteroids (tablets, suspension, or injection), or an increase from a stable maintenance dose, for at least 3 days, and a hospitalization or emergency department visit because of asthma requiring systemic corticosteroids. Exclusion criteria included respiratory infection, chronic obstructive pulmonary disease, vocal cord dysfunction, obstructive sleep apnea, Churg-Strauss syndrome, cardiac dysfunction, allergic bronchopulmonary aspergillosis, or poor adherence to treatment. The local research ethics committee of the Soonchunhyang University Hospital research board approved the study protocol.

Spirometry

Spirometry was performed before and after bronchodilator use.¹⁹ Baseline forced vital capacity (FVC) and FEV₁ measurements were obtained in the absence of bronchodilator use (within 8 hours). Basal and post-bronchodilator FEV₁ and FVC values were measured. A Vmax Series 2130 Autobox Spirometer (Sensor Medics, San Diego, California) was used and a calibration check was performed every morning at 8 AM.

Skin Prick Tests

Skin prick tests were performed using 55 common inhalant allergens, including dust mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), cat fur, dog fur, cockroaches, grass, tree, pollens, ragweed, and *Aspergillus* species (Bencard Co, Brentford, United Kingdom).²⁰ Atopy was defined as having a wheal reaction from the allergen equal to or larger than the histamine wheal (1 mg/mL) or at least 3 mm in diameter. Total IgE was measured using the UniCAP system (Pharmacia Diagnostics, Uppsala, Sweden).

Mediator Assays

Angiotensin-1 and Ang2 were assessed in plasma samples using enzyme-linked immunosorbent assays according to the manufacturer's instructions. Ang1 and Ang2 levels were checked during asthma control and before and during exacerbation. The minimum detectable limits of Ang1 and Ang2 were 1.36 to 10.3 and 1.20 to 21.3 pg/mL, respectively (R&D Systems, Minneapolis, Minnesota). Blood was drawn to evaluate serum albumin levels using laser nephelometry. All values were expressed in picograms per milliliter. For Ang1 and Ang2, the intra- and interassay variabilities were 3.2% and 5.8%, respectively.

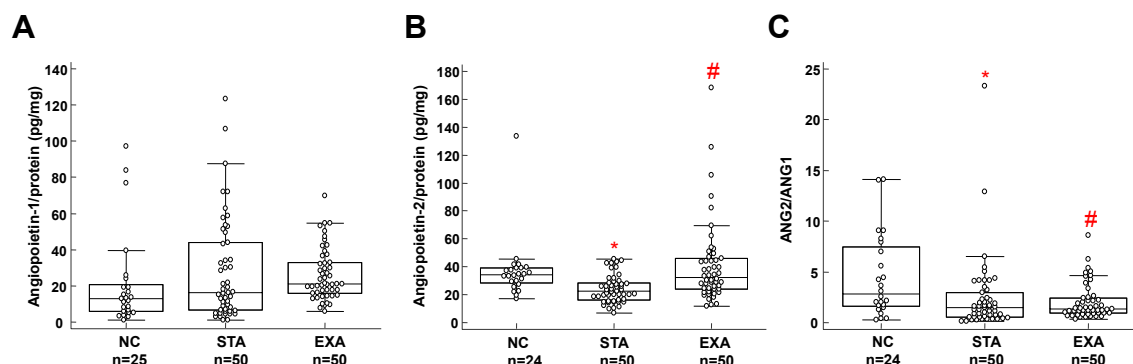


Figure 1. Circulating (A) angiotensin-1 (Ang1) and (B) and angiotensin-2 (Ang2) plasma levels and (C) Ang2/Ang1 ratio in patients with exacerbated asthma (EXA), patients with controlled asthma (STA), and healthy control subjects (NC). * $P < .01$ compared with healthy control subjects. # $P < .01$ compared with patients with controlled asthma.

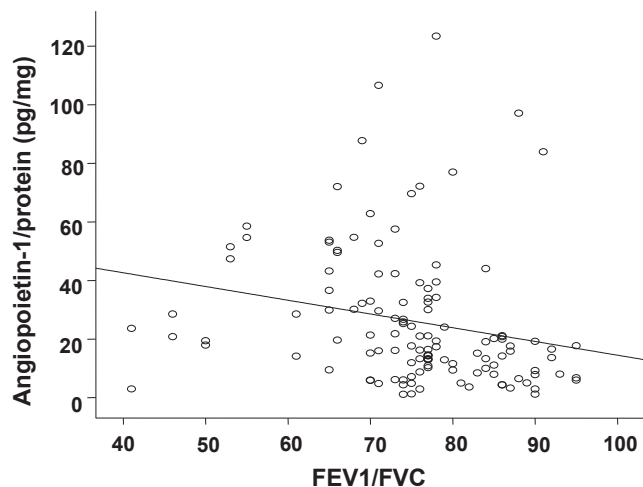


Figure 2. Correlation of angiotensin-1 with lung function in patients with asthma. FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity.

Statistical Analyses

Data were doubly entered into a statistical software package (SPSS 14.0; SPSS, Inc, Chicago, Illinois). Data are expressed as mean $\pm$ SEM. Group differences between patients with asthma and control subjects and between stable and exacerbated states in patients with asthma were compared using 2-sample *t* tests, Mann-Whitney *U* tests, or Pearson χ^2 tests for normally distributed, skewed, or categorical data, respectively. A *P* value less than .05 indicated statistical significance.

Results

Clinical Characteristics of Subjects

The clinical characteristics of 50 patients with asthma (mean age $\pm$ SD, 54.9 $\pm$ 14.1 years) and 25 control subjects (58.3 $\pm$ 6.2 years) were determined. The initial FEV₁ percentage of predicted values, FVC percentage of predicted values, and FEV₁/FVC were significantly lower in patients with asthma than in control subjects. Total IgE levels, presence of atopy, and blood eosinophil proportions were significantly higher in patients with asthma than in control subjects. Body mass index did not differ between patients with asthma and control subjects. The mean duration of disease in those with asthma was 6.63 $\pm$ 3.60 years, and the exacerbation rate per year during follow-up was 3.38 $\pm$ 3.24 (episodes per year). FEV₁ percentage of predicted values, FVC percentage of predicted values, and FEV₁/FVC were significantly lower in patients with exacerbated asthma than in those with controlled asthma. Blood neutrophil proportions were significantly higher in patients with exacerbated asthma than in patients with controlled asthma.

Circulating Angiotensin Levels as a Biomarker of Asthma

The mean plasma levels of Ang1 were 28.4 $\pm$ 4.01 pg/mg in patients with bronchial asthma and 21.2 $\pm$ 5.21 pg/mg in healthy controls (Figure 1). The mean plasma levels of Ang2 were 23.96 $\pm$ 1.38 pg/mg in patients with bronchial asthma and 36.8 $\pm$ 4.46 pg/mg in healthy controls (*P* = .010). The Ang2/Ang1 ratio was lower in those with asthma than in control subjects (2.45 $\pm$ 0.52 vs 4.47 $\pm$ 0.83, respectively; *P* = .046). Plasma Ang1 levels were correlated with FEV₁/FVC values (*r* = −0.257, *P* = .026) (Figure 2), whereas plasma Ang2 levels were correlated with FEV₁ percentage of predicted values (*r* = 0.309, *P* = .007), FEV₁/FVC ratio (*r* = 0.284, *P* = .014), and total IgE levels (*r* = −0.300, *P* = .009). The Ang2/Ang1 ratio was correlated with FEV₁ percentage of predicted values (*r* = 0.343, *P* = .003) and FEV₁/FVC ratio (*r* = 0.382, *P* = .001).

Circulating Angiotensin Levels as a Biomarker of Asthma Exacerbation

Although plasma Ang1 levels tended to be lower in patients with exacerbated asthma (26.1 $\pm$ 2.04 pg/mg) than in those with stable asthma (28.4 $\pm$ 4.01 pg/mg), Ang2 levels were higher in those with exacerbated asthma (41.0 $\pm$ 4.16 pg/mg) than in those with stable asthma (23.9 $\pm$ 1.38 pg/mg, *P* = .001). Lung function, as indicated by FEV₁ percentage of predicted and FEV₁/FVC ratio, decreased in patients with exacerbated vs controlled asthma.

Plasma Ang2 levels were correlated with initial eosinophil proportions (*r* = −0.449, *P* = .001) and initial neutrophil proportions (*r* = −0.337, *P* = .001) and with exacerbated eosinophil proportions (*r* = −0.566, *P* = .005; Figure 3). The Ang2/Ang1 ratio also was correlated with exacerbated eosinophil proportions (*r* = −0.655, *P* = .001; Figure 4).

Discussion

In this study, circulating Ang2 levels were increased in patients with exacerbated or uncontrolled asthma. Furthermore, Ang2 levels were correlated with severity of airway obstruction, indicating that circulating angiotensins could be an indicator of asthma exacerbation. Previous research has shown an increase in the size and number of bronchial blood vessels in patients with uncontrolled asthma, suggesting that angiogenesis is an essential component of tissue growth and remodeling in asthma.^{21–23}

Previous work has found that Ang1 is constitutively expressed in perivascular cells during development and in adult tissues, whereas Ang2 is expressed minimally or not at all in most normal adult tissues.²⁴ Functionally, Ang1 inhibits vascular permeability without affecting vascular morphology, whereas Ang2 might be involved in detaching smooth muscle cells and loosening the matrix.²⁴

Angiotensin-1 and Ang2 are endogenous ligands for the vascular endothelial tyrosine kinase receptor Tie2.²⁵ Signaling by Ang1 promotes vascular endothelial cell survival and the sprouting and reorganization of blood vessels and inhibits activation of the vascular endothelial barrier to decrease leakage and leukocyte

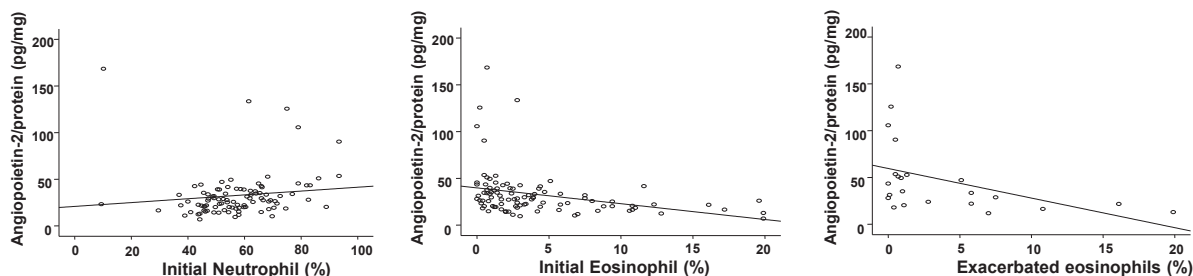


Figure 3. Correlation of angiotensin-2 with initial neutrophil proportions, initial eosinophil proportions, and exacerbated eosinophil proportions in patients with asthma.

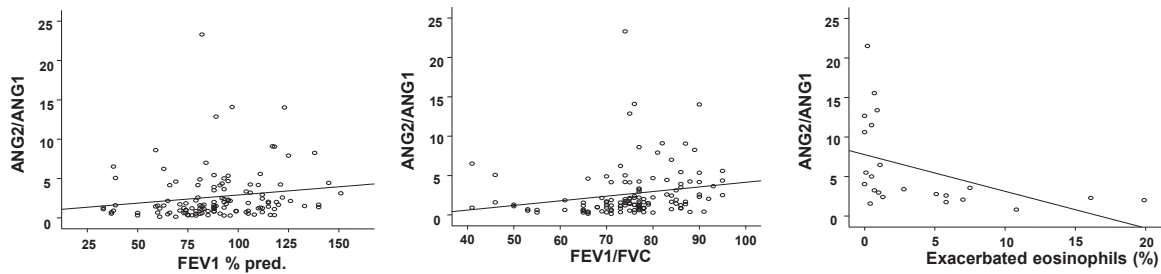


Figure 4. Correlation of ratio of angiopoietin-2 (Ang2) to angiopoietin-1 (Ang1) with lung function and exacerbated eosinophil proportions in patients with asthma. FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity.

migration into tissues.²⁵ Ang2 generally has an opposing action and is released naturally at times of vascular growth and inflammation.²³ Previous work has found that Ang1 levels are decreased in mice with asthma, prompting the authors to suggest that administration of Ang1 might be of therapeutic value because it prevents increased responsiveness of the airways to constrictors and ameliorates inflammation.²⁶ Increased Ang2 expression in airway epithelial cells and alveolar macrophages in mice with chronic asthma suggests that Ang2-induced, Tie2-mediated signaling might be the predominant signaling pathway.¹⁴

Asthma exacerbation increases vascular permeability, leading to pulmonary edema.²⁷ Inhaled furosemide exerts diuretic and anti-inflammatory effects by inhibiting the production and release of cytokines of inflammatory cells characteristic of respiratory tract diseases.²⁷ Angiopoietins are involved in other respiratory diseases, such as chronic obstructive pulmonary disease, acute lung injury, and lung cancer,^{9–11} and their expression differed from that in the present study. Further studies are needed to discriminate and clarify the role of angiopoietins. Moreover, targeting this pathway with novel therapeutic agents would improve asthma control and treatment.

In humans, circulating Ang2, vascular endothelial growth factor, and von Willebrand factor are increased during acute lung injury and acute respiratory distress syndrome and sepsis.¹³ In a previous study, increased Ang2 levels were correlated with impaired pulmonary gas exchange,²⁵ and circulating Ang2 levels were associated with pulmonary edema and severity of acute lung injury and acute respiratory distress syndrome.²⁸ In another study, serum Ang1 levels were higher in patients with asthma than in healthy subjects, and the serum Ang1/Ang2 ratio was correlated with lung function, suggesting that angiopoietins are useful markers for the diagnosis of asthma.¹⁶

In the present study, the level of circulating Ang2 was decreased in patients with stable disease compared with control subjects, whereas circulating Ang1 levels did not differ between healthy control subjects and those with controlled asthma. These results suggest, consistent with previous work, that Ang1 and Ang2 play different roles regarding the increase in pulmonary permeability leading to asthma.¹³ David et al¹⁴ reported increased Ang1 and Ang2 levels in the induced sputum of patients with severe refractory asthma, and Ang2 levels were positively correlated with the severity of airway occlusion. In contrast, the present study showed that Ang2 levels were lower in patients with controlled asthma, suggesting that Ang2 might differ according to target tissue (eg, blood vs airway epithelium). In this study, circulating Ang2 levels increased significantly in patients with exacerbated or uncontrolled asthma compared with the stable state, indicating that exacerbation of asthma can induce Ang2 expression, leading to an increase in the permeability of the lung. In addition, circulating Ang2 levels were positively correlated with lung function and negatively correlated with blood eosinophils and neutrophils in patients with stable asthma and those with exacerbated asthma.

These results suggest that Ang2 levels are decreased to recruit inflammatory cells, leading to less severe airway obstruction. In this study, lung function parameters such as FEV₁ percentage of predicted and FEV₁/FVC decreased in patients with exacerbated disease compared with those with controlled disease. Moreover, circulating Ang1 and Ang2 levels and the Ang2/Ang1 ratio were correlated with lung function, suggesting that angiopoietin-mediated signaling might influence asthma severity.

The limitation of this study was its small sample and, therefore, a lack of generalizability. Further studies with a large number of patients are needed to understand more fully the role of angiopoietin in asthma and its exacerbation.

In conclusion, although circulating Ang1 levels tended to be higher in patients with controlled asthma, Ang2 levels were significantly lower in those with controlled asthma. In addition, although plasma Ang1 levels tended to be lower in patients with exacerbated asthma, Ang2 levels were significantly higher in those with exacerbated asthma, suggesting that circulating angiopoietins could be a useful marker of asthma exacerbation.

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