

Elevated complement C3a in plasma from patients with severe acute asthma

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Background: Complement component C3a, an anaphylatoxin, provokes acute inflammatory responses, including smooth muscle contraction, mucus hypersecretion, increase in vascular permeability, and recruitment of inflammatory cells. Thus C3a may be related to airway inflammation and bronchoconstriction in acute asthma exacerbation.

Objective: We sought to determine whether plasma C3a is elevated in patients presenting for emergency treatment of acute asthma exacerbations and to correlate C3a concentrations with response to therapy.

Methods: Plasma C3a and serum eosinophil cationic protein were measured in 52 patients with acute asthma with peak expiratory flow of $\leq 50\%$ the predicted value. Control subjects were 42 patients with stable chronic asthma. Patients with severe acute asthma were classified into 2 groups (admitted and discharged), according to how effective inhaled bronchodilators and systemic corticosteroids were in the first 2 hours of treatment.

Results: Concentrations of C3a in plasma from subjects in the admitted group (median, 256 ng/mL; range, 94 to 454) were significantly higher than those in the discharged group (197 ng/mL; 72 to 300) or those in patients with stable chronic asthma (166 ng/mL; 89 to 254; $P < .0001$). Elevated plasma C3a concentrations in admitted asthmatic patients decreased significantly by 7 days after admission ($P = .0005$). No significant difference was evident in serum eosinophil cationic protein concentration between the admitted group (33.1 $\mu\text{g/L}$; 6.3 to 143) and the discharged group (32.7 $\mu\text{g/L}$; 14.6 to 160; $P = .99$).

Conclusions: Concentrations of C3a, which can induce airway inflammation and bronchoconstriction, were associated with differences in response to emergency treatment of severe asthma exacerbation. (*J Allergy Clin Immunol* 2003;112:525-30.)

Key words: asthma, innate immunity, complement, C3a, eosinophil cationic protein

International guidelines recommend multiple doses of inhaled β_2 -agonists and anticholinergic drugs plus early systemic administration of corticosteroids for treatment of acute severe asthma.¹ Responsiveness to therapy, however, varies widely between individuals. Recently we reported that in nearly one third of adults with acute severe asthma,

Abbreviations used

ANOVA: Analysis of variance
BAL: Bronchoalveolar lavage
C3aR: C3a receptor
ECP: Eosinophil cationic protein
PEF: Peak expiratory flow

such an intensive therapy protocol had little effect in the first 120 minutes.² These results underscored the need for better understanding of mechanisms of acute severe asthma exacerbation and more effective specific therapies.

Complement has a vital role in innate immunity. Several biological interactions in the complement pathway that are likely to participate in acute asthma involve the C3a anaphylatoxin, a potent proinflammatory mediator generated early in inflammatory reactions by proteolytic cleavage of complement C3.³ Many features of asthma exacerbations such as smooth muscle contraction, mucus hypersecretion, and recruitment of inflammatory cells are consistent with actions of C3a.⁴ In addition, C3a can stimulate the release of histamine and leukotrienes from basophils and mast cells as well as regulate synthesis of eosinophil cationic protein (ECP) by eosinophils.⁵⁻⁷ High potency, early timing, and the overall pattern of the reaction suggest involvement of C3a in the pathophysiology of asthma. Several recent reports have implicated complement anaphylatoxins as likely effectors in animal models or mildly asthmatic individuals.^{8,9}

These data support the hypothesis that in addition to acquired immune responses, the innate immune system, that is, the complement system, is involved in the pathogenesis of acute asthma. However, only a few reports have dealt with associations between complement activation and acute asthma exacerbation.¹⁰ We therefore determined whether plasma C3a concentrations were elevated in patients presenting for emergency treatment of severe acute asthma and correlated plasma C3a with response to this treatment. In addition, we measured serum concentrations of ECP, which has been reported to increase in association with acute asthma exacerbation,¹¹ to look for associations with plasma C3a and with therapeutic response.

METHODS

Fifty-two patients with an acute exacerbation of asthma who were treated between August 2000 and July 2001 in the emergency or outpatient department at Hamamatsu Rosai Hospital, a 400-bed urban

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TABLE I. Clinical characteristics of the 3 groups of patients

	Severe acute asthma		Stable asthma (n = 42)	P value
	Admitted (n = 24)	Discharged (n = 28)		
Age (y)	48 (16-76)	44 (16-72)	49 (28-59)	.25
Female (%)	70	68	57	.47
Current smoker (%)	17	36	19	.18
Duration of symptoms (y)	9 (1-46)	10 (1-48)	11 (1-45)	.29
Atopy (%)	67	85	76	.18
PEF (L/min)	143 (70-300)	183 (0-310)	365 (260-680)	<.0001
PEF % predicted	34 (17-50)	43 (0-50)	83 (67-108)	<.0001

Data are presented as median with range.

medical center, were enrolled in the study. All patients met the criteria for asthma proposed by the American Thoracic Society.¹² Our inclusion criteria were age between 18 and 75 years; an asthma history of at least 12 months; ability to perform an adequate forced expiratory maneuver; peak expiratory flow (PEF) of $\leq 50\%$ the normal predicted value; and a smoking history of <10 pack-years.

Patients were excluded from the study if they had chronic obstructive pulmonary disease, pneumonia, pneumothorax, acute myocardial infarction, or congestive heart failure; needed immediate resuscitation or airway intervention; had been treated with oral and/or intravenous corticosteroids the preceding 4 weeks; or were pregnant. Before enrollment, written informed consent was obtained from all patients.

Other participants in this study included 42 stable asthmatic subjects without symptoms of an exacerbation in the previous 3 months. The mean PEF of these 42 subjects at the time of enrollment was $>80\%$ of the normal predicted value. These subjects are designated here as having "stable asthma." The study was approved by the institutional review board.

Subjects with acute asthma exacerbations were treated with 4 puffs of salbutamol (Sultanol, GlaxoSmithKline, Tokyo, Japan; 100 μg per puff) every 20 minutes for 3 doses (total, 1200 μg), plus 4 puffs of oxitropium bromide (Tersigan, Nippon Boehringer Ingelheim, Hyogo, Japan; 100 μg per puff) combined with each salbutamol dose (total, 1200 μg). A metered-dose inhaler with a spacer device (InspirEase, Schering-Plough, Osaka, Japan) was used. A single dose of 125 mg of methylprednisolone (Solu-medrol, Pharmacia Upjohn, Tokyo, Japan) was administered intravenously to all patients at the time of the first dose of salbutamol.

Demographic data collected on entry were age, sex, and height. Clinical data including body temperature, respiratory rate, heart rate, accessory respiratory muscle use, dyspnea, wheezing, smoking history, asthma history, and usual asthma medications were recorded. Atopy was defined by at least one positive radioallergosorbent test score (CAP-RAST; Pharmacia and Upjohn Diagnostics, Uppsala, Sweden) to common inhalant allergens (*Dermatophgoides pteronyssinus*, animals, pollen, insects, *Alternaria*, *Aspergillus*, and *Candida*).

Pulse oximetry (Pulsox-M2; Minolta, Osaka, Japan) was recorded during breathing of room air, and oxygen was given as required. PEF was measured at baseline and at 20, 40, 60, and 120 minutes with the patient seated, using a mini-Wright peak flow meter (Clement Clarke, Harlow, UK). Trained nurses obtained the PEF, reading the highest of 3 values. Predicted values for PEF were based on data reported by Tsukioka.¹³

Patients with severe acute asthma were classified into 2 groups, according to response to treatment. After 120 minutes, patients were discharged (discharged group) if they were asymptomatic and free of accessory muscle use, had absent or diminished wheezing, were able to walk 20 m without exacerbation of symptoms and signs,¹⁴ and attained a PEF $>50\%$ of the predicted value.¹ Patients not meeting these criteria were hospitalized (admitted group).

Measurement of C3a/C3a des-arginine in plasma

Plasma was collected in EDTA-containing tubes at the time of presentation for emergency treatment of asthma exacerbation in the acute asthma group and at the time of enrollment in the stable asthma group. In the admitted group, plasma was sampled again 7 days after admission. Plasma was separated from cells within 1 hour and stored at -40°C until assay. We confirmed in advance that storage of plasma at -40°C up to 12 weeks did not affect measurement results for C3a concentration. C3a/C3a des-arginine were measured with the use of a radioimmunoassay kit (Amersham Pharmacia Biotech, Uppsala, Sweden). Interference from precursor C3a in the plasma sample was avoided by carrying out a selective precipitation step before assay. C3 in specimens was removed by the addition of a precipitating reagent. All samples were assayed undiluted, according to the protocol outlined by the manufacturer.

Measurement of ECP in serum

Serum was collected through the use of silicone-containing tubes (Vacutainer hemogard SST tubes; Becton Dickinson, Meylan, France). After clotting for 60 minutes at room temperature, venous blood samples were centrifuged at room temperature at 1000g for 15 minutes. Sera then were divided into aliquots and frozen at -40°C for subsequent assay of ECP with the use of a radioimmunoassay kit (Pharmacia and Upjohn Diagnostics). All samples were assayed undiluted, according to the protocol outlined by the manufacturer.

Statistical methods

Data are presented as median with range. Data were examined for statistical significance by Mann-Whitney *U* test for continuous variables. χ^2 or Fisher exact tests were used for categoric variables. The effects of improvements in PEF as a percentage of the predicted value were assessed by using repeated-measures analysis of variance (ANOVA). Comparison of clinical parameters, plasma C3a, and serum ECP among the 3 groups was made by the Kruskal-Wallis test. Differences among the study groups were assessed by means of post hoc pairwise multiple comparison with the Scheffé test. For all analyses, a 2-tailed *P* value of $<.05$ was considered indicative of statistical significance. Statistical analysis was performed with the StatView Statistical Package (Version 5.0; SAS Institute, Cary, NC).

RESULTS

Background data for patients with acute severe asthma and those with stable asthma who were enrolled in this investigation are summarized in Table I. Patients with acute severe asthma were classified into 2 groups (admitted and discharged), according to response to inhaled bronchodilators and systemic corticosteroids over a period

Asthma, rhinitis, other
respiratory diseases

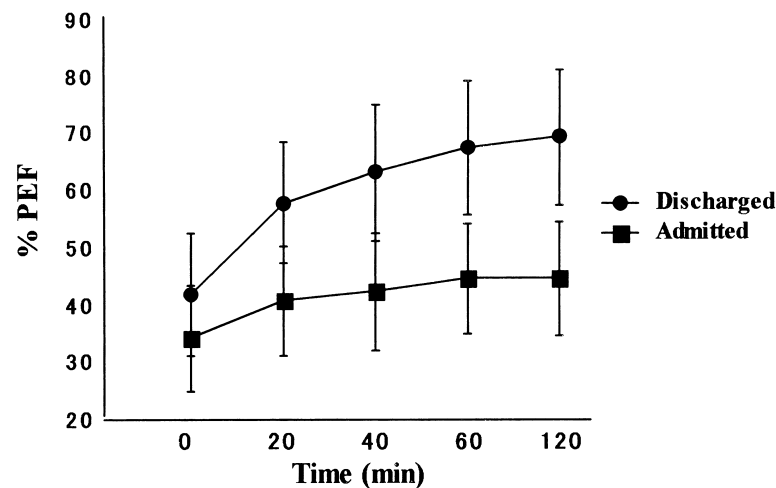


FIG 1. Changes in PEF from baseline. Significant difference in PEF response over time was present between admitted and discharged groups (ANOVA, $P < .0001$). Values are mean $\pm$ SD.

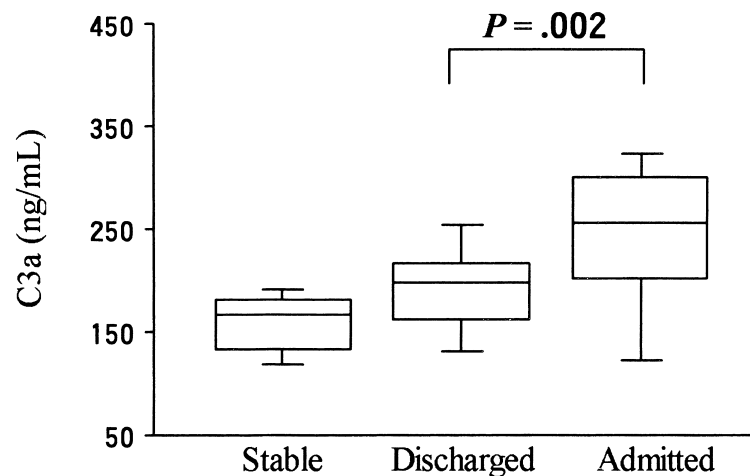


FIG 2. Comparison of plasma C3a concentration in patients with acute severe asthma (admitted and discharged group) and those with stable asthma. Significant difference was detected by Kruskal-Wallis test for comparison among the 3 groups ($P < .0001$). C3a showed a significant difference between the admitted and the discharged groups ($P = .002$). Values are presented as upper and lower quartiles and range (box), median value (horizontal line), and 95% CI (whisker line).

of 2 hours. A total of 94 patients were studied (24 admitted, 28 discharged, and 42 stable). The 3 groups did not differ significantly in age, sex, duration of asthma, smoking, or atopy. However, a significant difference was noted in both PEF and PEF as a percentage of the predicted value at presentation between the stable asthmatic patients and the 2 subgroups of patients with severe acute asthma ($P < .0001$). A highly significant difference in PEF response over time was present between the admitted and discharged groups (ANOVA, $P < .0001$; Fig 1). Discharged subjects showed an overall greater improvement in PEF (17.3%; 95% confidence interval, 10.9% to 23.3%; $P < .0001$) compared with admitted subjects.

The median plasma C3a concentration was 256 ng/mL (range, 94 to 454) in the admitted group, 197 ng/mL (range, 72 to 300) in the discharged group, and

166 ng/mL (range, 89 to 254) in subjects with stable asthma, representing a significant difference by the Kruskal-Wallis test for the comparison among the 3 groups ($P < .0001$). In addition, C3a showed a significant difference between the admitted group and the discharged group ($P = .002$, Fig 2). In the admitted group, plasma C3a also was compared between time of presentation for emergency treatment and 7 days after admission. Plasma C3a at 7 days after admission had decreased significantly in comparison with C3a at presentation (221 ng/mL; range, 123 to 278 vs 256 ng/mL; range, 94 to 454; $P = .0005$, Fig 3).

The median serum ECP concentration was 33.1 μ g/L (range, 6.3 to 143) in the admitted group, 32.7 μ g/L (range, 14.6 to 160) in the discharged group, and 19.3 μ g/L (range, 2.0 to 52.3) in subjects with stable asthma.

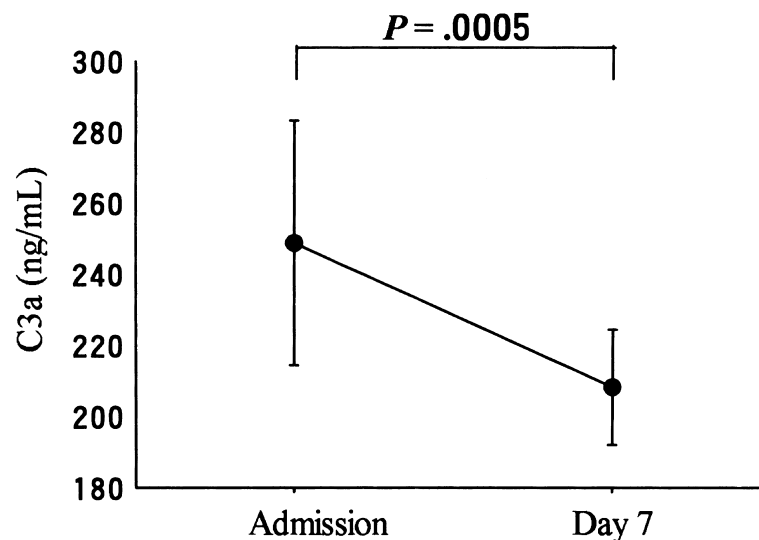


FIG 3. Plasma C3a concentrations in admitted patients at the time of presentation and 7 days after admission. Plasma C3a at 7 days after admission had decreased significantly compared with C3a at presentation ($P = .0005$). Values are mean $\pm$ SD.

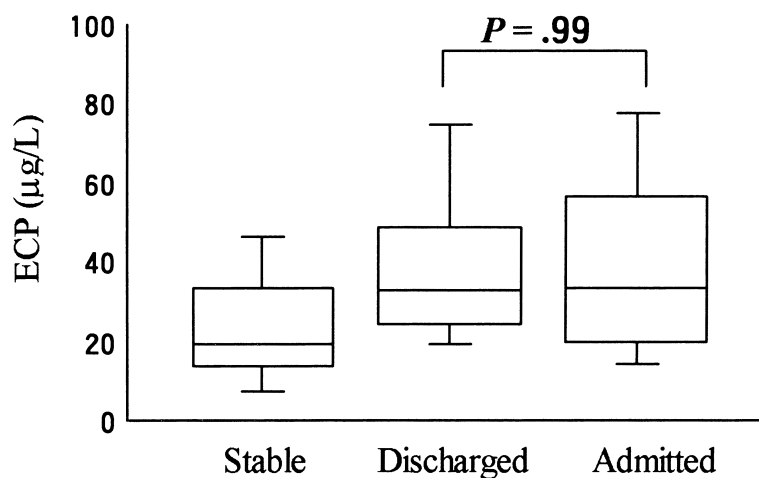


FIG 4. Comparison serum ECP in patients with acute severe asthma (admitted and discharged group) and those with stable asthma. Significant difference was detected by Kruskal-Wallis test for comparison among the three groups ($P < .0001$). No significant difference was noted between admitted and discharged groups ($P = .99$). Values are presented as upper and lower quartiles and range (box), median value (horizontal line), and 95% CI (whisker line).

A significant difference was detected by the Kruskal-Wallis test for the comparison among the 3 groups ($P < .0001$). However, no significant difference was noted in these concentrations between the admitted group and the discharged group ($P = .99$, Fig 4).

Patients who had inhaled β_2 -agonists before the emergency department visit accounted for 45% of patients requiring hospitalization and 41% of patients who were discharged, indicating no significant difference in the effect of inhaled β_2 -agonists between the 2 groups of patients. Therefore, we judged that therapy before the emergency department visit did not affect C3a and ECP measurements.

DISCUSSION

We documented that plasma C3a concentrations were significantly higher in patients seeking emergency treatment for severe asthma exacerbations than in control subjects with stable asthma. Plasma C3a concentrations were highest among patients who failed to improve during 2 hours of treatment with inhaled bronchodilators and intravenous corticosteroids. Elevations of plasma C3a in admitted asthmatic patients had decreased significantly at 7 days after admission. Taken together, these data suggest that plasma C3a may be involved in induc-

ing airway inflammation and bronchoconstriction and may reflect response to emergency treatment in severe asthma exacerbations. In contrast, no significant difference in serum ECP was seen between the admitted group and the discharged group.

International consensus guidelines recommend multiple doses of inhaled β_2 -agonists and anticholinergic drugs plus early systemic administration of corticosteroids for acute severe asthma.¹ However, nearly one third of patients with acute severe asthma are refractory to this intensive emergency treatment.² This suggests that multiple pathways may generate bronchospastic and/or inflammatory mediators in this clinical situation. A number of mediators have been suggested to participate in the effector phase of acute severe asthma, including cysteinyl leukotrienes,¹⁵ eotaxin,¹⁶ and eosinophil-derived granule proteins.¹⁷ When Di Lorenzo et al¹¹ evaluated serum ECP and bronchodilator response in acute asthma exacerbation, subjects with acute exacerbation of asthma showed high serum concentrations of ECP, whereas the response to bronchodilators was better in patients with lower serum ECP. We measured serum concentrations of ECP in patients with acute severe asthma. Although we found a significant difference in serum ECP between stable and severe acute asthmatic patients, ECP concentrations were similar between the admitted and discharged groups. Possible reasons for differences in results between the 2 studies include differences in severity of acute exacerbation (mean PEF: in our subjects, 38% of predicted value; in the subjects of Di Lorenzo et al, 59%) and differences in emergency treatment (in our patients, inhaled β_2 -agonists and anticholinergic drugs plus systemic corticosteroids; in theirs, inhaled β_2 -agonists alone).

The C3a anaphylatoxin is a potent pro-inflammatory mediator generated in the early phase of an inflammatory reaction by proteolytic cleavage of complement component C3.³ Many features of acute asthma, such as smooth muscle contraction, mucus hypersecretion, and recruitment of inflammatory cells, could be caused or worsened by the actions of C3a. Recently, several investigators have implicated complement anaphylatoxins as potential effectors in asthma. When Krug et al⁸ evaluated the role of anaphylatoxins in patients with mild asthma, they found a significant increase of C3a and C5a in bronchoalveolar lavage (BAL) fluid after allergen challenge compared with baseline values. Furthermore, they found a strong correlation between both anaphylatoxins and the number of eosinophils and neutrophils in BAL fluid and concluded that their data suggested contributions of anaphylatoxins C3a and C5a to acute asthma exacerbations. Humbles et al⁹ demonstrated that C3a receptor (C3aR)-deficient mice were protected against bronchoconstriction and airway hyperresponsiveness after allergen challenge. As for C5a, Teran et al¹⁸ investigated neutrophil chemotactic factors in BAL fluid from atopic asthmatic subjects, identifying C5a and interleukin-8 in BAL fluid of patients with asthma as major neutrophil chemotactic factors. Previously, C3aR was thought to be present only on myeloid cells such as

macrophages, neutrophils, and eosinophils.¹⁹ However, Drouin et al²⁰ recently demonstrated expression of C3aR on various human lung cells including bronchial epithelial cells, bronchial smooth muscle cells, alveolar cells, and pulmonary blood vessels. These findings suggest that in addition to IgE-mediated acquired immune responses, complement-mediated innate immune system responses may be involved in the pathogenesis of acute asthma.

Although previous reports have indicated that airway inflammation is prominent at the time of acute asthma exacerbation, the degree of airway inflammation cannot be quantified by invasive methods such as BAL or bronchial biopsy, especially during a severe exacerbation. In patients with severe airflow obstruction, techniques such as bronchoscopy with BAL and biopsy are precluded because they may worsen airway obstruction. Analysis of induced sputum is widely used in the evaluation of airway inflammation in mild to moderate asthma.²¹ Although safety and practicality of sputum induction in mild to moderate stable asthma have been well documented, data in patients with severe acute asthma are limited.²² Previous investigations have identified many significant changes in the lung.²³ Additionally, changes involving mediators and immunopathologic findings have been noted in peripheral blood from patients during acute asthma exacerbations, including elevated eotaxin concentrations¹⁶ and T-cell activation.²⁴ Recently, Jousilahti et al²⁵ analyzed associations of asthma with 3 sensitive markers of systemic inflammation: C-reactive protein, serum amyloid-A, and plasma fibrinogen. They concluded that markers of systemic inflammation, particularly serum amyloid-A and fibrinogen, were positively and significantly associated with prevalence of asthma. These findings suggest that inflammatory components contributing to asthma may include systemic factors as well as those limited to the lung.

Complement activation forms a central core of innate immune defense against mucosal viruses, fungi, bacteria, and other pathogens.²⁶ Viral respiratory tract infections commonly precede asthma exacerbations in both children and adults. Atmar et al²⁷ evaluated 148 episodes of asthma exacerbation in adults treated in the emergency department, finding viral respiratory tract infections in 50% of subjects who required hospitalization. In addition, Black et al²⁸ investigated the relation between positive skin tests for fungal spore allergy and admission to an intensive care unit for asthma. Subjects admitted to the intensive care unit were more likely to have a positive skin test result for one or more fungal allergens than patients not admitted to the intensive care unit. Mechanisms by which viral infection and sensitization to fungi provoke severe asthma exacerbations are not fully defined, but complement activation by viruses or fungi may be involved.

Several limitations to our study require consideration. Because the sample size of our study was small, we could not draw firm conclusions about how closely plasma C3a concentration was related to acute asthma. In this study, the effective rate of treatment, based on the guidelines, was 54%. To increase the effective rate of treatment

to 70% by administering anti-C3aR antibody therapy, that is, to achieve approximately a 15% improvement over the placebo group, 175 subjects are needed at the 5% significance level, with 80% power.²⁹ The methods did not allow us to distinguish whether C3a is involved directly as a mediator or is simply a marker for the acute severe asthmatic response. The definitive answer concerning whether C3a is important in the pathophysiology of acute severe asthma awaits availability of C3aR antagonists for clinical trials in patients with asthma.

Overall, our study demonstrated that plasma C3a was significantly higher in subjects with acute severe asthma than in control subjects with stable asthma and that plasma C3a concentrations were highest among patients who initially failed to respond to inhaled β_2 -agonists and anticholinergics plus administration of systemic corticosteroids. Despite many recent advances in acute asthma management, morbidity and mortality remain high. The search for better treatment strategies for improving lung function and reducing hospital admission rates in acute severe asthma must continue.

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