

ORIGINAL ARTICLE

Comparison of Serum Total Antioxidant Status Between Patients with Community Acquired Pneumonia and Severe Asthma Exacerbation

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ABSTRACT

BACKGROUND: Oxidant/antioxidant imbalance has been reported in various respiratory diseases including pneumonia and asthma. However, the role of blood antioxidants has not been fully analyzed.

OBJECTIVES: The aim of the present study was to measure and compare serum total antioxidant status (TAS) in patients with community-acquired pneumonia (CAP) or asthma exacerbation during hospitalization.

METHODS: Forty-five patients (41 men – 4 women, with a mean age of 49±22 years) admitted to our hospital with community-acquired pneumonia and 30 patients with severe asthma exacerbation (22 men – 8 women, with a mean age of 41.3±20.7 years), as well as 18 normal subjects (40.2±10 years of age) were included in the study. On admission and on the seventh day, serum TAS was measured using a colorimetric method at 600 nm.

RESULTS: In both groups TAS on admission was decreased compared with normal subjects (0.83±0.13 vs. 1.19±0.09 mmol/L, $p<0.001$ and 0.98±0.08 vs. 1.19±0.09 mmol/L, $p<0.001$ respectively). In patients with CAP, but not in asthmatics, TAS on discharge was still decreased compared with normals (1.00 ±0.18 vs. 1.19±0.09 mmol/L, $p<0.001$). Change of TAS between the first and seventh day was not different between the two groups of patients. Comparison between TAS on admission and TAS on discharge revealed statistically significant difference in both groups of patients (asthma: 0.98±0.08 vs. 1.13±0.18 mmol/L, $p<0.001$, pneumonia: 0.83±0.13 vs. 1.00±0.18 mmol/L, $p<0.001$). Comparison of TAS on admission and TAS on discharge between the two groups of patients showed that both measurements were significantly decreased in patients with pneumonia compared with asthmatics (0.83±0.13 vs. 0.98±0.08 mmol/L, $p<0.001$ and 1.00±0.18 vs. 1.13±0.18 mmol/L, $p=0.003$ respectively). Decreased TAS on admission was found in smokers with pneumonia compared with smokers/asthmatics but on discharge this difference was obscured. Non-smokers with pneumonia had decreased TAS both on admission and discharge compared with non-smokers /asthmatics.

CONCLUSIONS: Serum TAS is decreased in patients with CAP or asthma exacerbation suggesting the presence of oxidative stress. Although TAS change during the course of the disease is similar, this decrease is more profound in CAP patients on admission as well as on discharge.

INTRODUCTION

Involvement of oxygen free radicals has been associated with a number of pulmonary diseases such as the adult respiratory distress syndrome, bronchopneumonic dysplasia, emphysema, pneumoconiosis, hyperoxia, bleomycin toxicity, cystic fibrosis, bronchial asthma, pneumonia, etc. The source of oxidants varies considerably for each specific case.^{1,2} Oxygen free radicals are inactivated by antioxidant mechanisms which include enzymes such as superoxide dismutase, catalase, glutathione peroxidase and others such as albumin, uric acid, lactoferrin, β -carotene, vitamins C and E, etc.^{3,4}

Increased generation of reactive oxygen species in vivo can lead to the depletion of one or more antioxidants and loss of individual antioxidants can be measured as an index of oxidative stress.⁵ Over the recent years, several attempts have been made to assess the total antioxidant activity of body fluids rather than specifically identifying what has happened to each component of the complex antioxidant defence system.^{6,7} There is evidence that interaction between inflammation and oxidative stress or antioxidants exists independently of the origin of inflammation.^{8,9} Pneumonia and asthma are both inflammatory diseases although totally different with regards to the nature of the underlying inflammation; pneumonia is characterized by acute inflammation associated with infection as opposed to asthma which is considered to be a chronic non infectious inflammatory disease.

We have previously shown that serum total antioxidant status (TAS) was decreased in patients with severe exacerbation of asthma or community acquired pneumonia and correlated with the severity of the disease.^{10,11} The aim of the present study was to compare TAS between patients with community-acquired pneumonia (CAP) or severe asthma exacerbation during hospitalization in an attempt to investigate the response of the antioxidant mechanisms of two different inflammatory diseases to increased oxidative burden.

PATIENTS AND METHODS

Forty-five patients (41 men – 4 women, with a mean age of 49.0 ± 22.1 years) admitted to our hospital for community-acquired pneumonia and 30 patients with severe asthma exacerbation (22 men – 8 women, with a mean age of 41.3 ± 20.7 years) were studied. Eighteen normal subjects (40.2 ± 10.0 years of age) were also included in the study. Patients' characteristics are summarized in **Table 1**.

Pneumonia was defined as an acute infection of lung parenchyma presenting radiologically as a new consolidation on chest X-ray accompanied by fever, cough with or without purulent or mucopurulent sputum, with or without increased white blood cell count (WBC). Admission criteria were based

TABLE 1. Patients' Characteristics

	Pneumonia	Asthma
Number	45	30
Age (years)	49 ± 22.1	41.3 ± 20.7
PaO ₂ * (mmHg)	72.90 ± 14.27	62.90 ± 10.74
PaO ₂ ** (mmHg)	79.90 ± 14.80	85.20 ± 10.57
WBC*	13576 ± 5972	8956 ± 2650
WBC**	8764 ± 1353	7485 ± 1753
FEV ₁ * (L/sec)	-	1.47 ± 0.71
FEV ₁ ** (L/sec)	-	2.6 ± 1.12
Smokers (pack years)	29 (18 ± 12.3)	10 (15 ± 17.5)

FEV₁= forced expiratory volume at one second; PaO₂= partial oxygen pressure; WBC= white blood cell count

*on admission; **on discharge

upon the CURB index (presence of acute confusion, urea nitrogen >7 mmol/l, respiratory rate ≥ 30 /min, blood pressure $<90/60$ mmHg) and patients with a CURB ≥ 2 were admitted to the hospital.¹² Sputum Gram stain, sputum culture, blood and/or pleural fluid culture were performed for the detection of the etiological agent. During the study, all patients received antibiotics (amoxicillin+clavulanic acid, a macrolide, second or third generation cephalosporin with or without an aminoglycoside in immunocompromised patients) separately or in combination.¹³ Twenty-nine pneumonia patients were current smokers and 16 had pneumonia predisposing factors (heart failure, n=10; chronic obstructive pulmonary disease, n=4; immunosuppression, n=2).

Diagnosis of asthma was based on symptoms and reversibility of airflow limitation by bronchodilators according to GINA guidelines.¹⁴ None of the asthmatic patients had a history of atopy defined as giving a positive wheal and flare response to skin prick testing with several common allergens and ten of them were current smokers. All patients were on inhaled corticosteroid administration prior to the study. The inclusion criteria were based upon low forced expiratory volume in one second (FEV₁) ($<60\%$ predicted) or severe hypoxemia (PaO₂ <60 mmHg (8 kPa)) or presence of at least one severity criterion such as need to be seated, difficulty to speak, use of accessory respiratory muscles, respiratory rate >30 /min, pulse rate >120 /min, pulsus paradoxus and also lack of clinical improvement despite intensive medical treatment at the emergency department. The cause of the exacerbation of their disease was upper or lower respiratory tract infection based upon clinical criteria. All patients were treated with nebulised bronchodilators (beta₂-agonists and ipratropium bromide) as well as oral or parenteral steroids, at the same dose, and/or

antibiotics occasionally. The discharge criteria were based upon the combination of improved PaO_2 (>70 mmHg (9.3kPa)), normal respiratory rate and pulse rate, clinical improvement and/or improvement of FEV_1 ($>60\%$).

In both groups of patients none was receiving oral steroids for at least one month prior to the study entry or any kind of medication with known antioxidant properties, such as trimetazidine, non-steroidal anti-inflammatory drugs such as acetylsalicylic acid, nimesulide, etc. Supplemental oxygen was given in concentrations no more than 40% to avoid increased production of oxidants due to hyperoxia. Patients with severe hypoxemia demanding a higher than 40% oxygen concentration were excluded from the study. On admission and on the 7th day, blood samples were drawn for complete blood count and serum chemistries. Arterial blood was drawn for the measurement of partial oxygen pressure (PaO_2) on room air. The study received the approval of the institutional committee on human experimentation and all subjects gave written informed consent.

COLLECTION OF BLOOD SAMPLES AND TAS MEASUREMENT

On admission and on the 7th day, 5 ml of venous blood sample was centrifuged in 3000 rpm for 10 minutes. Samples were collected before supplemental oxygen was administered. Serum was removed and stored at -70°C . A kit from Randox Ltd (Crumlin, Co Antrim, UK) was used for the measurement, based on a colorimetric method suitable for serum samples.¹⁵ In this method, incubation of 2,2'-azinobis-3-ethylbenothiazoline 6-sulphonate (ABTS[®]) with a peroxide (metmyoglobin) results in production of the radical cation ABTS^{+•}. This species is blue-green in color, and can be detected at 600 nm. Antioxidants in the added sample cause inhibition of this color production to a degree that is proportional to their concentration. A Dimension DU PONT-DADE analyzer was used for the measurement and the reagent was calibrated with the standards contained in the kit.

STATISTICAL ANALYSIS

Results are expressed as mean \pm SD. Paired t-test was used for the comparison in measurements of antioxidants between the first and seventh day. One way ANOVA was used to compare TAS on the first and seventh day between normal subjects, pneumonia and asthmatic patients. The Bonferonni post hoc test was used to establish differences among groups. Unpaired t-test was used to compare TAS values between smokers and non smokers in pneumonia and asthmatic patients. The Statistical Program for Social Science (SPSS version 17, Chicago, IL) was used for the analysis.

RESULTS

PATIENTS

The etiological agent was identified in 18 patients (40%) based mainly on positive blood cultures or pleural fluid cultures. The most common bacteria were *Streptococcus pneumoniae* (10 cases) and Gram (-) (*Haemophilus influenzae*, *Branhamella catarrhalis*, *Klebsiella* and only one case of *Acinetobacter* in immunocompromised patient). None from the two groups of patients studied had to be admitted to the intensive care unit.

TOTAL ANTIOXIDANT STATUS (TAS)

In both groups (patients with pneumonia and asthmatics), TAS on admission was decreased compared with normal subjects (0.83 ± 0.13 vs. 1.19 ± 0.09 mmol/L, $p < 0.001$ and 0.98 ± 0.08 vs. 1.19 ± 0.09 mmol/L, $p < 0.001$ respectively) (Figure 1). In asthmatic patients, TAS on discharge was not statistically different compared with normal subjects (1.13 ± 0.18 vs. 1.19 ± 0.09 mmol/L, NS). However, in patients with pneumonia, TAS on discharge was still decreased compared with normals and this was statistically significant (1.00 ± 0.18 vs. 1.19 ± 0.09 mmol/L, $p < 0.001$) (Figure 1).

Change of TAS between the first and seventh day was not significantly different between the two groups of patients (0.18 ± 0.20 vs. 0.15 ± 0.14 , NS). Comparison between TAS on admission and TAS on discharge revealed statistically significant difference in both groups of patients (asthma: 0.98 ± 0.08 vs. 1.13 ± 0.18 mmol/L, $p < 0.001$, pneumonia: 0.83 ± 0.13 vs. 1.00 ± 0.18 mmol/L, $p < 0.001$) (Figure 2).

Comparison of TAS on admission and TAS on discharge between the two groups of patients showed that both measurements were significantly decreased in patients with pneumonia compared with asthmatics (0.83 ± 0.13 vs. 0.98 ± 0.08 mmol/L, $p < 0.001$ and 1.00 ± 0.18 vs. 1.13 ± 0.18 mmol/L, $p = 0.003$ respectively) (Figure 1).

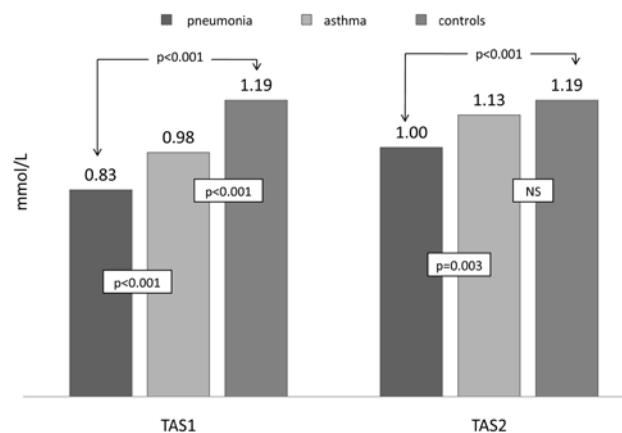


FIGURE 1. TAS differences among three groups of subjects.

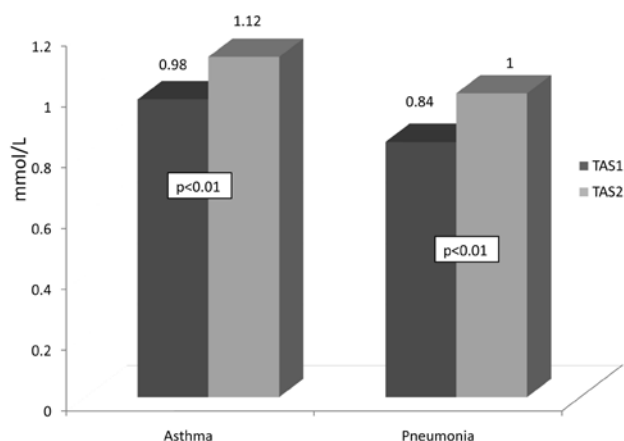


FIGURE 2. TAS differences on admission and discharge.

When the patients were evaluated according to smoking habit, no statistically significant differences were observed on admission between smokers and non smokers, both in asthma and pneumonia patients. Decreased TAS on admission was found in smokers with pneumonia compared with smokers/asthmatics (0.81 ± 0.13 vs. 0.97 ± 0.07 mmol/L, $p < 0.001$). However, on discharge this difference was obscured (0.99 ± 0.17 vs. 1.09 ± 0.12 mmol/L, NS). On the other hand, non-smokers with pneumonia had decreased TAS both on admission and discharge compared with non-smokers/asthmatics (0.87 ± 0.13 vs. 0.99 ± 0.10 mmol/L, $p = 0.01$ and 1.01 ± 0.20 vs. 1.18 ± 0.22 mmol/L, $p = 0.04$ respectively) (Figure 3).

DISCUSSION

The present study demonstrated a significant decrease of

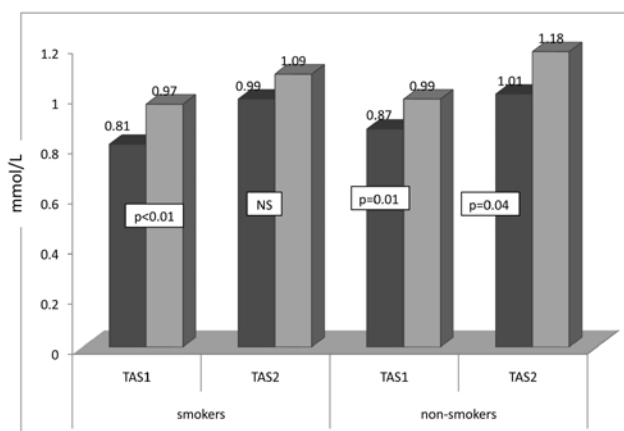


FIGURE 3. TAS differences between smokers and non smokers.

serum TAS in patients with severe exacerbation of asthma or community acquired pneumonia, compared to healthy subjects, indicating the presence of oxidant/antioxidant imbalance, probably due to increased oxidative load. This imbalance has been recently supported by further studies regarding both pneumonia and acute exacerbations of asthma. For example, increased oxidative stress while decreased enzymic and non-enzymic antioxidant activities were demonstrated in children with acute pneumonia.¹⁶ Increased serum levels of reactive oxygen metabolites were found in patients with acute exacerbation of asthma^{17,18} and they decreased after treatment with systemic steroids and bronchodilators.¹⁷

Even though the natural time course of TAS in respiratory diseases has not been investigated to date, serum TAS increased after a week of appropriate treatment in both groups of patients following improvement of the clinical and laboratory findings. According to clinical experience, most of pneumonia patients show improvement in clinical and laboratory findings within a week period. Thus, we arbitrarily chose the seventh day to estimate TAS changes in order to correlate these changes with pneumonia improvement.

Comparison of TAS measurements in both groups of patients revealed significantly lower values in pneumonia patients as opposed to the asthmatics. A plausible explanation could be the role of neutrophils through the oxidative burst phenomenon. It is known that the oxidative response of activated phagocytes is a primary host defense mechanism in pneumonia and that this response is mainly due to neutrophil recruitment and activation.¹⁹ Furthermore, the host defense against *Streptococcus pneumoniae* which is a major cause of pneumonia depends on bacterial killing by neutrophils.²⁰

However, there is evidence that at sites of inflammation, multiple inflammatory cells including eosinophils, neutrophils and macrophages are capable of generating reactive oxygen species which can contribute to development of various diseases.²¹ Especially in asthma, the numbers of inflammatory cells - particularly eosinophils - are increased in the airways and release large amounts of harmful reactive oxygen species.²² Furthermore, increased cell numbers of activated eosinophils and cells with respiratory burst activity in peribronchial tissue and peripheral blood have been detected in unstable asthma.²³

It seems that the different nature of inflammation that characterizes CAP and asthma is not enough to explain the differences in TAS values between these diseases, as both neutrophils and eosinophils are major sources of reactive oxygen species. It is possible that the subacute and more prolonged course of pneumonia leads to greater antioxidant reduction through increased oxidative load which is not restored even at the recovery period.

A limitation of our study is that oral or parenteral steroids were given as treatment in asthmatic patients as opposed to pneumonia patients who did not receive steroids and this could have had an impact on the increase of TAS. However,

lack of administration of oral or parenteral steroids in patients with severe asthma exacerbation is beyond medical ethics. Furthermore, in the present study no statistically significant differences in TAS changes during the seven-day period were found between the two groups of patients, supporting the aforementioned hypothesis as both groups showed similar improvement in antioxidant status but in CAP patients the initial values were much lower compared to the asthmatics. In addition, pneumonia may be related to lower TAS due to the fact that there is a more prominent effect observed in peripheral circulation. However, there was no significant difference in TAS between patients with positive blood culture and those without, although the number of those patients was too small (3 cases) to provide reliable statistical results.

Smoking habit appeared not to have any influence on the oxidative stress in pneumonia and asthmatic patients on admission. On the other hand, smokers with pneumonia or asthma had no difference in TAS on discharge. A plausible explanation could be that usually smokers quit smoking during hospitalization.

Measurement of serum total antioxidant status obviously offers an overall estimation of the extracellular antioxidant capacity. That means that probably most of the antioxidants which take part in the colorimetric method used in the present study are extracellularly located, although there is evidence that major intracellular antioxidant enzymes (e.g. SOD and GSH-Px) are also detectable extracellularly even in smaller amounts.²⁴

CONCLUSION

The results of our study strongly suggest the presence of a profound antioxidant deficiency, through decreased serum TAS, in asthmatic patients with severe exacerbation of their disease or in patients with community acquired pneumonia. Although asthma and pneumonia are pathophysiologically totally different diseases, they are both characterized by inflammation. However, the relationship between the decrease in serum TAS and the pathogenesis of acute exacerbations of asthma or the course of CAP requires further study, as well as the benefit of supplementary administration of antioxidants.

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