Clin Chem Lab Med 2007;45(10):1326–1331 © 2007 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2007.268

Angiotensin-converting enzyme (ACE) I/D corrected serum ACE activity and severity assessment of community-acquired pneumonia

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Abstract

Background: Various studies have described decreased serum angiotensin-converting enzyme (ACE) activity in patients with pneumonia. The aim of the present study was to evaluate the role of ACE in pneumonia by comparing ACE insertion/deletion (I/D) genotype corrected serum ACE activity and to establish whether the severity of the disease correlates with lower ACE activity.
Methods: This was a prospective hospital-based observational study including 134 patients with pneumonia. Serum ACE activity was determined at admission, on days 2, 3, 5 and 10 of hospital stay, and at recovery. Based on ACE genotype and reference values, corresponding Z-scores were calculated. Disease severity, quantified by the acute physiology score (APS), and clinical outcome were compared between tertile groups of the Z-scores.
Results: A significant decrease in serum ACE activity during an episode of pneumonia with return to control range during recovery was observed for all three genotypes (II, ID and DD). The calculated Z-scores showed a negative correlation with APS scores ($p=0.050$). No significant association between decreased serum ACE activity and clinical outcome was observed.
Conclusions: Serum ACE activity is significantly decreased during the acute phase of pneumonia. Despite correction for ACE I/D genotype, decreased ACE activity did not show a prognostic value. Further studies are needed to examine the mechanisms behind and diagnostic value of decreased ACE activity in community-acquired pneumonia.
Clin Chem Lab Med 2007;45:1326–31.

Keywords: angiotensin-converting enzyme; I/D polymorphism; outcome assessment; pneumonia.

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Introduction

Community-acquired pneumonia remains a major reason for hospital admission and a common cause of death in developed countries (1, 2). Therefore, pneumonia is subject to many studies on demographic variables, comorbidities and biological markers in order to predict outcome and to evaluate a patient's management.

The renin-angiotensin system is a feedback regulated system. In response to a fall in blood pressure, renin is secreted into the circulation. Renin cleaves angiotensinogen to generate angiotensin I. Angiotensin I has no appreciable activity, but is acted on by a second proteolytic enzyme, angiotensin-converting enzyme (ACE; peptidyldipeptidase A, EC 3.4.15.1) to form the highly active angiotensin II. The majority of ACE is expressed on the surface of (pulmonary) endothelial cells (3) and leaked into the circulation from ACE-expressing cells by proteolytic cleavage (4). Circulating concentrations of ACE have been extensively studied in relation to different human lung disorders (5, 6). Today, one quite established value of serum ACE measurements is in the diagnosis and follow-up of sarcoidosis (7, 8). Furthermore, serum ACE has also been studied in patients with adult respiratory distress syndrome (ARDS) and pneumonia, where ACE activity showed a strong decrease in the acute phase of the disease with return to control range within a few days (9–13).

Although proposed, so far no relation between these alterations in serum ACE activity and clinical development of both ARDS and pneumonia could be established (14). One possible explanation for this could be the large inter-patient variations in serum ACE activity observed in these studies. Nowadays, the identified ACE insertion/deletion (I/D) polymorphism, accounting for almost half of the variance in serum ACE activity, provides an explanation for these inter-patient variations, but also a need to re-investigate

the possible clinical value of serum ACE activity in pneumonia by considering the ACE I/D genotype (15).

The aim of the present study was to evaluate the role of ACE in community-acquired pneumonia by comparing the genotype corrected serum ACE activity and to establish whether the severity of the disease correlates with ACE activity.

Materials and methods

The study was conducted in St. Antonius Hospital, a 600-bed teaching hospital (Nieuwegein, The Netherlands). The ethnicity of the population in and around the city of Nieuwegein is primarily white-Caucasian (>94%) (16).

Patient population

This was a prospective observational study of patients with confirmed pneumonia admitted between October 1, 2004 and August 1, 2006. Pneumonia was defined as a new or progressive infiltrate on a chest X-ray and at least two of the following criteria: cough, sputum production, temperature >38°C or <35°C, ausculatory findings consistent with pneumonia, leukocytosis or leukopenia (>10 g/L, <4 g/L or >10% rods in leukocyte differentiation), C-reactive protein more than three-fold greater than the upper limit of the reference interval for normal values. Patients who were immunocompromised [systemic steroid use at admission (prednisone equivalent >20 mg/daily for more than 3 days), haematological malignancies and other immunosuppressive therapy] or who were using ACE-inhibitors, angiotensin II receptor blockers or aldosterone antagonists were excluded. All patients were required to sign an informed consent form and the study was approved by the Ethics Committee of St. Antonius Hospital. In total, 158 patients with pneumonia were included in the study. For 24 of these patients, no blood sample for ACE activity measurement was collected at the time of hospital admission and an appropriate DNA sample was missing for one patient. A total of 134 patients were eligible for further analysis.

Determination of ACE activity

Blood samples were collected aseptically into lithium heparin tubes on admission and on days 2, 3, 5 and 10 of hospital stay. At least 30 days after the resolution of the acute infection, the patients were requested to visit the out-patient clinic to provide another blood sample. Quantification of ACE activity was measured in lithium heparin plasma using the Bühlmann ACE kinetic test, according to previously described methods (Bühlmann Laboratories AG, Allschwill, Switzerland) (17, 18). The manufacturer's reference interval is 12–68 U/L.

Genotyping

Genomic DNA of patients was isolated from EDTA blood using a MagNA Pure LC DNA Isolation kit 1 (MagNA Pure; Roche Diagnostics, Basel, Switzerland). ACE I/D polymorphisms were determined by real-time PCR using fluorescent hybridisation probes and a LightCycler (Roche Diagnostics) as described previously with some slight modifications (15, 19, 20). Briefly, the reaction volume was 20 μL, containing 1 μL of DNA (40–80 ng), 0.2 μM forward primer and 0.8 μM reverse primer reported by Rigat et al. (15), 2 μL of 10× reaction buffer (LightCycler DNA master hybridisation probes, Roche Diagnostics), 1.6 μL of 25 mM $MgCl_2$ stock solution and 0.1 μM of each probe. The detection probes were the same as described by Somogyvari et al. (20). The PCR conditions were as follows: denaturation at 95°C for 60 s, followed by 50 cycles with denaturation at 95°C for 10 s, annealing (first 10 cycles: at 67°C for 20 s, followed by a 0.5°C stepwise decrease per cycle to 61°C) and extension at 72°C for 30 s. Melting curve analysis consisted of heating to 95°C for 5 s, maintaining at 45°C for 60 s, followed by an increase in temperature to 75°C at 0.2°C/s. To exclude mistyping of I/D heterozygotes as D/D homozygotes, a second PCR reaction was conducted under the same conditions except for using the primer pair as described earlier (19, 20). Verification of the real-time PCR results with those of electrophoresis and using sequence-specific primer PCR revealed no mistyping. ACE I/D polymorphisms were determined after follow-up of the patients.

Genotype corrected ACE activity

After genotyping, all serum ACE activities were translated into Z-scores. The Z-score was calculated as $(ACE_{patient} - \text{mean } ACE_{reference\ group})/SD_{reference\ group}$, where $\text{mean}_{reference\ group}$ and $SD_{reference\ group}$ are calculated from the ACE values measured in a previously described II, ID and DD reference group originating from the same geographical region as the patients (21). This reference group consisted of healthy employees of St. Antonius Hospital who volunteered for venipuncture.

Pathogen identification

At least two blood cultures were performed and sputum was taken for Gram-stain and culture and analysed by Taqman real-time PCR for *Mycoplasma pneumoniae*, *Legionella pneumophila* and *Chlamydophila psittaci* (22). Pharyngeal samples were taken for viral culture. Urine was sampled for antigen testing on *Streptococcus pneumoniae* and *L. pneumophila* (Binax NOW®, Scarborough, Maine, USA) (23, 24). In addition, serum samples taken on the day of admission and day 10 were analysed in pairs for detection of a four-fold rise of antibodies to respiratory viruses, *Coxiella burnetii*, *M. pneumoniae* and *C. psittaci* by complement fixation assay (25). Based on the findings, patients were classified as having pneumonia with bacterial origin, viral pneumonia or pneumonia with unknown aetiology.

Outcome measures and illness severity assessment

The following outcome measures were identified for all patients: duration of hospital stay, the need for intensive care unit (ICU) admittance, survival to hospital discharge, and 28-day mortality. Illness severity was quantified by the acute physiology score (APS) on admission and consecutively on days 2, 3, 5 and 10 of hospital stay (26).

Comorbidity assessment

As well as demographic variables, comorbidities were identified to address factors related to the outcome of community-acquired pneumonia. Comorbidities were defined based on the presence of conditions for which the patient was under active medical supervision or was receiving treatment at the time of hospital admission. Comorbidities evaluated were lung diseases [chronic obstructive pulmonary disease (COPD) or treated asthma], congestive heart failure, diabetes (type I and type II) and end-stage renal disease (serum creatinine >150 μmol/L). Furthermore, patients were classified

Table 1 Demographic and general characteristics of the study population.

Characteristic	n = 134
Demographics	
Age, years, mean (SD)	61 (19)
Male sex, n (%)	79 (59)
Comorbidity	
Renal disease, n (%)	8 (6)
CHF, n (%)	10 (8)
Diabetes, n (%)	17 (13)
Lung diseases, n (%)	48 (36)
ACE genotype (II, ID, DD), n	32, 63, 39
Risk class[a]	
Low I, n (%)	25 (19)
Low II, n (%)	24 (18)
Low III, n (%)	30 (22)
Moderate IV, n (%)	40 (30)
High V, n (%)	15 (11)

[a]Risk class based on Fine et al. (27).

according to the pneumonia severity index developed by Fine et al. (27). In this index patients are classified into five categories representing predicted mortality (with the fifth category as highest mortality risk).

Statistical analysis

Statistical analysis was performed using the SPSS statistical package (version 12.0.1 for Windows; SPSS, Chicago, IL, USA). Continuous data were expressed as mean ± SD or median (interquartile range), where appropriate. Categorical data were analysed using the χ^2-test and continuous data using Student's t-test, rank tests and one-way analysis of variance, where appropriate. To study the association between serum ACE and severity of disease, a correlation coefficient between Z-score and APS was calculated. Furthermore, a multivariate linear regression analysis was performed to identify determinants for serum ACE activity (using ACE activity at recovery). Subsequently, a linear regression analysis was conducted with APS as dependent and serum ACE activity and all parameters that appeared independent determinants for serum ACE activity included in the model. The prognostic usefulness of serum ACE activity was studied in two ways. First, clinical outcomes were compared between tertile-based groups of the distribution of the Z-scores. Secondly, a statistical analysis focused on the ability of serum ACE activity to predict the outcome of pneumonia. For this purpose, logistic regression models adjusted by the comorbidities, ACE genotype and including serum ACE activity were constructed. The relative risk of having a certain outcome was estimated by odds ratio and 95% confidence interval. For all tests, a p-value <0.05 was considered statistically significant.

Results

The mean age of the patients was 61 years (±19 years) and 79 (59%) of them were male (Table 1). On the day of hospital admission, the serum ACE activity was significantly different for the II, ID and DD genotype groups with mean serum ACE activities of 24, 28 and 39 U/L, respectively (Table 2). In total, 96 patients visited the out-patient clinic for a recovery sample. The reason for not visiting was either death (n = 8) or lost to follow-up (n = 30). The ACE genotype distribution did not differ between both time points (n = 32, 63 and 39 on admission and n = 25, 48 and 23 on recovery for II, ID and DD, respectively; p = 0.733) and the genotype distribution was similar to that of the reference group (n = 43, 107 and 50, respectively; p = 0.356). When analysed as a paired-sample (n = 96), the serum ACE activity differed significantly between admission and recovery in all three genotype groups (Table 2). The decrease in serum ACE activity was most evident in carriers of the DD genotype. Figure 1 shows the serum ACE activity on the day of admission, on days 2, 3, 5 and 10 of hospital stay and at recovery for the three genotype groups. The serum ACE activity of the reference group is also presented in Figure 1. The mean serum ACE activities at hospital admission were 29, 29 and 34 U/L for pneumonia with bacterial origin (n = 81), viral pneumonia (n = 12) and pneumonia with unknown aetiology (n = 41), respectively.

The overall median duration of hospital stay was 9 days and 14 patients were admitted to the ICU. During hospital stay eight (6%) patients died, all due to pneumonia. Based on the calculated Z-scores, the patients were divided into three groups of equal size (based on tertiles). This resulted in groups with Z-scores from −3.2 to −1.2 (n = 46), from −1.2 to −0.4 (n = 43) and from −0.4 to 3.2 (n = 45). Duration of hospital stay, in-hospital mortality and 28-day mortality, as well as ICU admittance was not statistically different between the three groups (Table 3). However, there was a trend towards increased disease severity with lower Z-scores. The mean APS score was the highest in the group of patients with lowest Z-scores, and APS showed a negative correlation with the calculated Z-scores (p = 0.050). In the multivariate linear regression analysis, serum ACE was not significantly associated with APS (p = 0.156). Serum ACE activity at recovery was significantly determined by ACE genotype and lung diseases (COPD and asthma). In the multivariate logistic regression analyses, no associa-

Table 2 Serum angiotensin-converting enzyme (ACE) activity (U/L) according to the ACE genotype on admission and during recovery.

	II	ID	DD	p-Value[c]
ACE acute (n = 134)	24 (9)	28 (12)	39 (15)	<0.001
ACE recovery (n = 96)[a]	27 (7)	37 (12)	52 (17)	<0.001
p-Value[b]	0.019	<0.001	<0.001	

Data are mean (SD). [a]Number of patients in acute and recovery groups varied in size because not all patients visited the out-patient clinic as a recovery sample (8 patients died and 30 were lost to follow-up). [b]Paired-sample t-test (n = 96). [c]One-way analysis of variance.

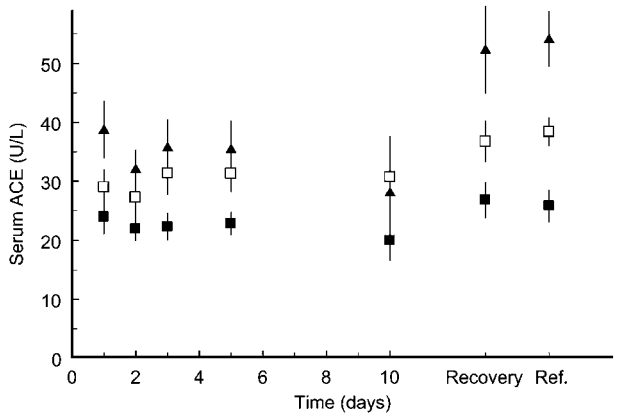

Figure 1 Mean serum angiotensin-converting enzyme (ACE) activity with 95% confidence intervals on admission (day 1) and on days 2, 3, 5 and 10 of hospital stay and after recovery for different genotypes.
■, II; □, ID; ▲, DD. The last column represents the ACE activity of the reference (Ref.) group.

tions between serum ACE activity and need for ICU admittance, in-hospital mortality or 28-day mortality could be detected, as the serum ACE activity did not reach significance in any of the models. The ACE I/D polymorphism also showed no association with need for ICU admittance and both in-hospital and 28-day mortality (data not shown).

Discussion

In accordance with the findings of Kerttula and Weber (13) and Al'tshuler et al. (28), our study showed a significant decrease in serum ACE activity during an episode of community-acquired pneumonia with return to control range during recovery. The decrease was evenly pronounced in pneumonia with bacterial origin, viral pneumonia and pneumonia with unknown aetiology. Despite correction for ACE I/D genotype and a significant correlation with the APS, we were not able to establish a prognostic value for the decreased ACE activity on the outcome of pneumonia.

The pathophysiological mechanisms behind the decrease in serum ACE activity are still unclear. Considering that ACE in the peripheral blood is identical to that produced by the pulmonary endothelial cells, one possible explanation could be an attenuated enzyme release from damaged pulmonary vascular endothelium. The observation from Al'tshuler et al. that ACE activity decreased more in patients with polysegmented pneumonia is supportive to such a mechanism (28). Another possible explanation could be an increased demand for angiotensin II leaving a depleted ACE pool. Hilgenfeldt et al. observed higher angiotensinogen levels combined with lower ACE levels in patients with sepsis (29). The fact that in the present study serum ACE activity also correlated with mean arterial pressure (used in the calculation of the APS) is supportive to such a mechanism (26). A third explanation could be the concomitant presence of circulating endogenous inhibitors. This, however, was studied by Al'tshuler et al. but not observed.

In addition to the studies by Al'tshuler et al. (28) and Kertulla and Weber (13), we included the ACE I/D polymorphism in the association between decreased serum ACE activity and severity of pneumonia, but we were still unable to show a single prognostic value for decreased serum ACE activity during the active phase of pneumonia. However, although non-significant, our findings show a trend towards a negative correlation between ACE activity and disease severity. Based on these findings, we believe that further studies with larger numbers are warranted to explore a prognostic value for decreased ACE activity in community-acquired pneumonia. However, we realize that pneumonia severity is multifactorially determined which could preclude clinical significance for the decreased ACE activity itself. The severity and clinical outcome remain strongly determined by demographic patient characteristics and comorbidities, as well as the aetiology of pneumonia and antibacterial treatment. To further study the mechanism responsible for the observed decreased serum ACE activity during an episode of pneumonia, concomitant angiotensin II sampling would be helpful.

Although in the present study we were unable to establish a prognostic value for the decreased ACE activity in the acute phase of pneumonia, there may be a diagnostic applicability. To explore this possibility, we compared the serum ACE activity at admission with those of the healthy control subjects used for our calculation of the Z-scores. For example, considering a cutoff point of 52 U/L for patients with the DD genotype in deciding on the diagnosis of pneumonia, ACE activity showed a sensitivity of 52% and a specificity of 90%. These data indicate that an ACE

Table 3 Clinical outcomes and illness severity by Z-score tertiles (Z-score calculated based on serum angiotensin-converting enzyme activity at the time of hospital admission).

Severity and outcome	Z-score			p-Value
	(−3.2 to −1.2) n=46	(−1.2 to −0.4) n=43	(−0.4 to 3.2) n=45	
APS, mean (SD)	22 (14)	19 (10)	17 (12)	0.111
Duration of stay, median (interquartile range)	10 (6–15)	9 (7–14)	8 (6–15)	0.908
ICU admittance, n (%)	5 (11)	3 (7)	6 (13)	0.618
In-hospital mortality, n (%)	3 (7)	2 (5)	3 (7)	0.906
28-day mortality, n (%)	3 (7)	2 (5)	3 (7)	0.906

APS, acute physiology score; ICU, intensive care unit.

activity above this cutoff point practically excludes the diagnosis of pneumonia. However, we realise that such a diagnostic applicability requires further study, especially because this was not the primary aim of the present study. Furthermore, serum ACE activity of initially suspected pneumonia that is not confirmed at follow-up will also need to be assessed.

There are some possible limitations to our study. As it was conducted in admitted patients, less severe episodes of pneumonia attended normally in the primary care setting could not be included, although 59% of the episodes were grouped into the low risk classes (class I–III). Secondly, one could argue about the sample size of the present study. Beforehand, no solid power calculation was conducted as a decrease in serum ACE activity was not predictable. Moreover, the mixed character (e.g., different aetiologies) of the study population could have weakened the power to detect an association between serum ACE activity and the outcome of pneumonia. In a post hoc power calculation, our study had sufficient power to detect a three-fold increased in-hospital mortality for patients with a Z-score below -2 compared to patients with higher Z-scores ($\alpha = 0.05$, power $(1-\beta) = 0.80$). Another possible limitation is the lack of information about smoking status. Smoking is associated with both an increased risk of pneumonia and increased plasma ACE levels (30). Therefore, smoking could modify the decrease in serum ACE activity during pneumonia. Unfortunately, it was not possible to evaluate this in the present study. Finally, we observed a decrease in circulating concentrations of ACE, but this may not necessarily represent a decrease in ACE levels in the lung. Although ACE is mainly derived from endothelial cells and the lung represents the body's largest endothelial surface, in ARDS patients an increased ACE activity has been reported in bronchoalveolar lavage fluid, despite a decrease in circulating concentrations (31).

In conclusion, serum ACE activity is significantly decreased during the acute phase of pneumonia with return to normal during recovery. Despite correction for ACE I/D genotype, the decrease in ACE activity did not show a prognostic value. Further studies are needed to examine the mechanisms behind and diagnostic value of decreased ACE activity in community-acquired pneumonia.

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Received March 26, 2007, accepted May 21, 2007