Prognostic Value of Plasma Human β-Defensin 2 Level on Short-Term Clinical Outcomes in Patients With Community-Acquired Pneumonia: A Preliminary Study

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BACKGROUND: Plasma level of human β-defensin 2 (HBD-2), noted to play a role in lung inflammatory diseases, is elevated in patients with pneumonia. OBJECTIVE: To investigate the prognostic value of plasma HBD-2 concentration in predicting 30-day clinical outcomes in patients with community-acquired pneumonia (CAP). METHODS: Patients with CAP were divided into 2 groups, based on the 30-day clinical outcomes, presence or absence of adverse outcomes (death, need for invasive mechanical ventilation, development of new complication of pneumonia). Demographic data, comorbidities, baseline clinical and laboratory features, plasma HBD-2 concentration, and the CURB-65 (confusion, urea nitrogen, breathing frequency, blood pressure, ≥ 65 years of age) scores on admission were compared between the 2 groups in univariable analysis. Multivariable logistic regression was used to test the predictor of adverse outcomes. Receiver operating characteristic analyses were used to calculate the power of the assays to predict the 30-day adverse outcomes. RESULTS: We enrolled 361 subjects with CAP between March 2007 and March 2011. Univariate analysis revealed the following as predictive factors: age, smoking status, duration from symptom onset to admission, bilateral radiographic changes, total white-blood-cell count, serum sodium, serum potassium, serum albumin, plasma HBD-2 concentration, CURB-65 score, and comorbidities. In the multivariable logistic regression, plasma HBD-2 concentration, CURB-65 score, and age were independent predictors of 30-day adverse outcomes. In the receiver operating characteristic analysis, plasma HBD-2 concentration had an area under the curve of 0.77 (95% CI 0.71–0.82); the optimal cutoff point was 12.5 mg/L (sensitivity of 63%, specificity of 84%, positive predictive value of 42%, and negative predictive value of 88%), which predicted 30-day adverse outcomes in subjects with CAP. CONCLUSIONS: In CAP patients, plasma HBD-2 level on admission is associated with 30-day clinical outcomes, and lower plasma HBD-2 level is an independent predictor for adverse outcomes. Plasma HBD-2 level may become a useful tool for prognostic stratification in patients with CAP. *Key words: β-defensins; prognosis; community-acquired pneumonia; CAP.* [Respir Care 2013;58(4):655–661. © 2013 Daedalus Enterprises]

Introduction

The protection of the human respiratory organs, directly in contact with the environment through the respiratory tract, is safeguarded through a very sophisticated defense system that has improved through the evolutionary process of the natural and acquired immune system. Defensins, small, cationic host-defense peptides that exert their protective functions at the host-environment interface, were

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initially proposed to act as innate immune effectors by a direct, antibiotic-like activity, and were recently found to function as signaling molecules via alerting and/or activating cellular components of innate and adaptive immunity[1,2] At least 2 related defensin families have been identified in humans: α defensins are expressed in neutrophils and certain macrophages, whereas β defensins are mostly produced by epithelial cells, especially in the respiratory tract epithelium.[3] Human β-defensin 2 (HBD-2), a cysteine-rich cationic amino acid antimicrobial peptide,[4] is significantly up-regulated by different stimuli such as proinflammatory cytokines and lipopolysaccharide[5] in the respiratory tract epithelium. Elevated HBD-2 displays chemotactic activity for CD8+T cells and triggers a robust production of cytokines for peripheral blood mononuclear cells.[6-8] The majority of prior studies focused on the pathophysiological effect of HBD-2 in respiratory infection, demonstrating activation of HBD-2 biosynthesis and release into the airway and plasma via bacterial or foreign microorganisms, and inflammatory cytokines stimulation following pneumonia or other lower-respiratory-tract infection.[9-16] However, few studies have examined the clinical implication of the elevated HBD-2 in community-acquired pneumonia (CAP), and whether HBD-2 predicts clinical outcomes in CAP. Therefore, this study evaluated the association between plasma HBD-2 concentration on admission and 30-day clinical outcomes in patients with CAP.

QUICK LOOK

Current knowledge

The plasma level of human β-defensin 2 (HBD-2) plays a role in inflammatory lung diseases and is elevated in patients with pneumonia.

What this paper contributes to our knowledge

In patients with community-acquired pneumonia, the admission plasma HBD-2 level was associated with 30-day clinical outcomes. Lower HBD-2 was an independent predictor for adverse outcomes. HBD-2 may be a tool for prognostic stratification in patients with community-acquired pneumonia.

Methods

To ensure comparability between treatments, this prospective study, performed in a satellite teaching hospital with 1,346 beds, was planned to include patients managed by the same medical team only. The inclusion criteria included:

- Age 18 years or older
- Presented with lower-respiratory-tract symptoms and signs consistent with pneumonia, including one or more of: new onset cough with or without sputum production, dyspnea, hemoptysis, pleuritic chest pain, new onset confusion, pyrexia, or altered breath sounds on chest auscultation
- Chest x-ray showing new infiltrates compatible with the presence of acute pneumonia

Exclusion criteria are listed in Table 1. The study was approved by the ethics committee, and informed consent was obtained from all subjects.

Demographics; smoking status; the duration of symptoms from onset to admission; empiric antibiotics used upon admission; comorbidities (including presence of cardiovascular disease, cerebrovascular disease, chronic renal failure, and diabetes mellitus); physical examination at baseline; and laboratory data on admission (including complete blood count, electrolytes, liver and kidney function tests, quantitative D-dimer, urine analysis, and electrocardiogram) were collected. Severity of CAP at presentation was assessed using the British Thoracic Society's severity of illness index CURB-65 (confusion, urea nitrogen, breathing frequency, blood pressure, ≥ 65 years of age).[17] Plasma HBD-2 concentration was measured with the enzyme-linked immunosorbent assay method on admission. The end points being evaluated for the 30-day clinical outcomes assessment included death; need for invasive mechanical ventilation; development of new complications (lung abscess, empyema, or complicated parapneumonic effusion); recovery (including complete recovery, substantial improvement); and persistent disease other than the status mentioned above. Death, need for invasive mechanical ventilation, or/and a new complication of pneumonia were categorized as adverse outcomes, indicating poor prognosis. Follow-up evaluation 30 days after admission, whether as in-patient or out-patient, was performed to confirm the final diagnosis of CAP and the absence of all exclusion criteria.

All statistical analyses were performed using statistics software (SPSS 15.0, SPSS, Chicago, Illinois). Data are expressed as means ± SD for continuous variables, and as frequencies or percentages for descriptive and clinical variables. One-factor analysis of variance was used for the comparison of quantitative variables, and chi-square test was used for the comparison of qualitative variables. In addition, the number of adverse outcomes using the quartiles of HBD-2 levels were gathered to assess the quantitative relationships between the HBD-2 levels and the adverse outcomes. If HBD-2 was associated with adverse outcomes in the univariable analysis, multinomial logistic regression was used to test whether HBD-2 was a predictor of adverse outcomes. In the logistic regression, all other baseline characteristics that showed association (or a

Table 1. Study Exclusion Criteria

Hospital-acquired or healthcare-acquired pneumonia
Pregnancy
Active thoracic or extrathoracic malignancy
Conditions likely to cause diagnostic confusion or where chest radiograph changes are equivocal (eg, pulmonary fibrosis, suspected active tuberculosis, Churg-Strauss syndrome, allergic bronchopulmonary aspergillosis)
Chronic lung disease (COPD, bronchiectasis, asthma)
Immunosuppression (iatrogenic or acquired)
Solid organ transplant
Hematological disorders, including hematological malignancy
Chronic liver disease or cirrhosis
Prolonged bed rest or clinical manifestations of aspiration
Acute comorbidities that would make pneumonia severity assessment inappropriate (eg, acute pulmonary embolism)
Patients for whom active treatment is not appropriate (palliative care)

trend toward it) with adverse outcomes were also included. Areas under the receiver operating characteristic curves and cutoff points for admission plasma HBD-2 levels were calculated, which provided the optimal positive and negative predictive values for adverse outcomes on the basis of the Youden index. For all analyses, a 2-tailed P value $< .05$ was considered statistically significant.

Results

Subjects' Characteristics on Admission and Adverse Outcomes in 30 Days

A total of 361 subjects were enrolled to the study between March 2007 and March 2011. Subjects' characteristics on admission are shown in Table 2. All subjects had been treated with oral or parenteral antibiotics, ranging from several hours to days prior to admission. Subjects were given the standard of care in accordance with standard guidelines[18] during hospitalization by the same medical team. Other accompanying therapies, including expectorant, Chinese herbal medicine, and short acting β_2-receptor agonist, were given based on presentation of the disease while immunologic or biologic agents were not allowed.

Eighty-seven subjects had adverse outcomes at the 30-day follow-up, including 13 (3.6%) deaths, 39 (10.8%) cases of invasive mechanical ventilation, and 60 (16.6%) cases of development of new complications. Thirty-five subjects were both in need of invasive mechanical ventilation and developed new complications.

A significant trend of correlation between crude 30-day adverse outcomes was noted among the quartile groups of HBD-2 concentration (Figure). Further analyses of the new complications showed that unadjusted parapneumonic pleural effusion, empyema or other migratory abscess, and other complications significantly correlated with HBD-2 plasma concentration using the interquartile categories (Table 3). ICU stay and duration of mechanical ventilation are shown in Table 4. There were no missing data.

Univariate Analysis of Plasma HBD-2 Concentration and Other Parameters for Adverse Outcomes

All the parameters listed in Table 2 were included in the univariate analysis to identify the predictive factor for adverse outcomes for the study population. Predictive factors that were of statistical significance included plasma HBD-2 concentration, age, smoking status, the duration from symptom onset to admission, bilateral radiographic changes, total white-blood-cell counts, serum sodium level of > 145 mmol/L or < 135 mmol/L, serum potassium level of > 5.0 mmol/L or < 3.2 mmol/L, serum albumin level of < 25 g/L, CURB-65 score, and comorbidities (see Table 2).

HBD-2 Concentration as a Predictor of Adverse Outcomes in the Multivariable Model

In the multivariable logistic regression, plasma HBD-2 concentration, CURB-65 score, and age were independent predictors of 30-day adverse outcomes: plasma HBD-2 concentration: odds ratio 0.77, 95% CI 0.70–0.85, $P < .001$; CURB-65 score: odds ratio 4.08, 95% CI 2.76–6.02, $P < .001$; age: odds ratio 1.05, 95% CI 1.01–1.10, $P = .03$) (Table 5).

Further analysis was carried out to assess the association between plasma HBD-2 concentration and CURB-65 scores on admission, and the Pearson product-moment correlation coefficient was -0.12, $P = .02$.

Predictive Value of HBD-2 for Adverse Outcomes in the Receiver Operating Characteristic Analysis

In the receiver operating characteristic analysis to determine the predictive value of HBD-2 for adverse outcomes, the area under the receiver operating characteristic curve was 0.77 (95% CI 0.71–0.82, $P < .001$). The cutoff points of plasma HBD-2 concentration and their sensitivity, specificity, positive and negative predictive values, and Youden index are shown in Table 6. Plasma HBD-2 concentration of 12.5 mg/L was the optimal cutoff value for adverse outcomes, as provided by the maximal Youden index.

Discussion

CAP remains a common lower-respiratory-tract infection in clinical practice and may result in serious compli-

Table 2. Subject Characteristics on Admission

	Patients With Adverse Outcomes $n = 87$	Patients Without Adverse Outcomes $n = 274$	P
Age, mean ± SD y	75.2 ± 6.6	69.6 ± 11.7	< .001
Male, no. (%)	50 (57.5)	155 (56.6)	.90
Smoking status, no. (%)			
Current or ex-smoker	59 (67.8)	140 (51.1)	.01
Nonsmoker	28 (32.2)	134 (48.9)	.01
Marital status, no. (%)			
Married	58 (66.7)	198 (72.3)	.34
Single/divorced/widowed	29 (33.3)	76 (27.7)	.34
Duration of symptoms before admission, mean ± SD d	8.7 ± 4.5	6.7 ± 3.5	.02
Radiographic bilateral changes, no. (%)	73 (83.9)	194 (70.8)	.02
Laboratory findings			
Hemoglobin, mean ± SD g/L	95.7 ± 19.5	113.7 ± 21.9	.37
Total white blood cell count, mean ± SD × 10^9 cells/L	101.1 ± 37.9	112.9 ± 55.9	.05
Serum sodium > 145 mmol/L or < 135 mmol/L, no. (%)	36 (41.4)	86 (31.4)	.06
Serum potassium > 5.0 or < 3.2 mmol/L, no. (%)	33 (37.9)	80 (29.2)	.08
Serum albumin < 25 g/L, no. (%)	26 (29.9)	56 (19.5)	.05
Serum D dimer, mean ± SD mg/L	0.57 ± 0.61	0.69 ± 0.68	.11
Plasma HBD-2, mean ± SD mg/L	9.1 ± 3.3	13.4 ± 5.0	< .001
CURB-65 score, mean ± SD	3.6 ± 1.1	2.3 ± 0.9	.02
Comorbidities, no. (%)			
Cardiovascular disease	52 (59.8)	145 (52.9)	.27
Cerebrovascular disease	32 (36.8)	76 (27.7)	.07
Chronic renal insufficiency	19 (21.8)	37 (13.5)	.05
Diabetes mellitus	39 (44.8)	93 (33.9)	.05
Two or more comorbidities	45 (51.7)	107 (39.1)	.03

HBD-2 = human β-defensin 2
CURB-65 = confusion, urea nitrogen, breathing frequency, blood pressure, ≥ 65 years of age

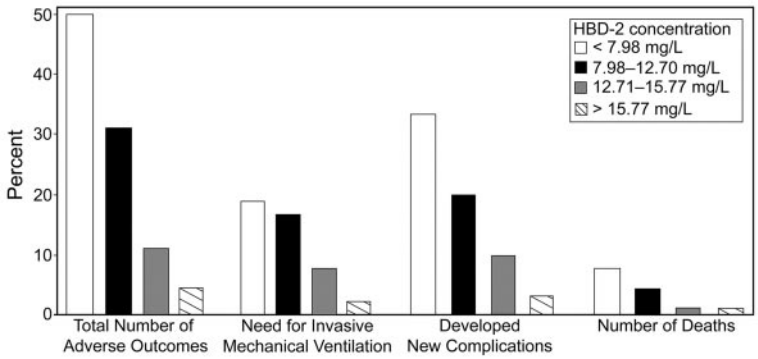

Figure. Crude 30-day adverse outcomes by quartiles categories of human β-defensin 2 (HBD-2).

cations or death, despite the availability of potent new antimicrobials and effective vaccines.[19] Severity scores and indices, developed to assist in clinical decision-making, have also been evaluated as prognosis predictors. In the past decade, application of the CURB-65 scoring system was often recommended in risk stratification and management for patients with CAP.[20] However, the prognosis of patients with CAP may be influenced not only by the initial severity of the disease but also many other factors, which may include but are not limited to host immune state.

HBD-2, with its strong bactericidal activity, plays a major role in the respiratory airway defense system, and modulates respiratory infection via its broad antibiotic spectrum against both Gram-negative and Gram-positive bacteria, fungi, and enveloped viruses by limiting bacterial

Table 3. Unadjusted New Complications of 30-Day Adverse Outcomes by HBD-2 Quartile

Plasma HBD-2 Quartile (mg/L)	Parapneumonic Pleural Effusion	Lung Abscess	Empyema or Other Migratory Abscess	Other*
< 7.98	27	8	9	9
7.98–12.70	13	6	5	8
12.71–15.77	5	3	2	3
> 15.77	3	1	1	0
P	< .001	.08	.02	.01

* Other: septic shock, multi-organ dysfunction syndrome, disseminated intravascular coagulopathy.
HBD-2 = human β-defensin 2

Table 4. ICU Stay and Mechanical Ventilation Time by HBD-2 Quartile

Plasma HBD-2 Quartile (mg/L)	ICU Stay mean ± SD d	Mechanical Ventilation Time mean ± SD h
< 7.98	13.6 ± 8.8, *n* = 28	161 ± 72, *n* = 16
7.98–12.70	11.7 ± 7.8, *n* = 19	150 ± 83, *n* = 19
12.71–15.77	12.5 ± 8.1, *n* = 6	148 ± 58, *n* = 3
> 15.77	13.0 ± 8.0, *n* = 3	161 ± 0, *n* = 1
P	.63	.75

HBD-2 = human β-defensin 2

Table 5. Predictors of Adverse Outcomes in the Multivariable Model

	Odds Ratio	95% CI	*P*
Age	1.05	1.01–1.10	.02
Smoking status	1.60	0.78–3.30	.20
Duration of symptoms before admission	1.09	0.99–1.20	.06
Total white blood cell count	1.07	0.98–1.16	.13
Radiographic bilateral changes	1.99	0.85–4.64	.11
Serum sodium > 145 mmol/L or < 135 mmol/L	1.69	0.84–3.40	.15
Serum potassium > 5.0 mmol/L or < 3.2 mmol/L	1.49	0.71–3.11	.29
Serum albumin < 25 g/L	2.16	0.95–4.90	.07
Plasma HBD-2	0.77	0.70–0.85	< .001
CURB-65 score	4.08	2.76–6.02	< .001
Cerebrovascular disease	1.25	0.51–3.09	.63
Chronic renal insufficiency	1.16	0.46–2.93	.75
Diabetes mellitus	1.15	0.51–2.59	.73
Two or more comorbidities	2.04	0.73–5.75	.18

HBD-2 = human β-defensin 2
CURB-65 = confusion, urea nitrogen, breathing frequency, blood pressure, ≥ 65 years of age

Table 6. Sensitivity, Specificity, Positive Predictive Values, and Negative Predictive Values for Adverse Outcomes With HBD-2 Cutoff Points

HBD-2 Cutoff (mg/L)	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Youden Index
7.0	0.90	0.39	0.32	0.92	0.29
8.5	0.81	0.53	0.35	0.90	0.34
10.0	0.69	0.68	0.41	0.87	0.37
11.5	0.69	0.72	0.44	0.88	0.41
12.0	0.69	0.76	0.48	0.89	0.45
12.5	0.63	0.84	0.42	0.88	0.47
13.0	0.56	0.86	0.56	0.87	0.42
14.5	0.39	0.93	0.64	0.83	0.32
16.0	0.31	0.97	0.77	0.82	0.28

HBD-2 = human β-defensin 2

adherence and invasion in respiratory infection.[21] A previous study[9] showed that plasma HBD-2 concentration increased in the acute stage of pneumonia, and that HBD-2 transcript product corresponded to the increases in both lung tissue and bronchoalveolar lavage fluid. When challenged by bacterial membrane and cell wall components or certain inflammatory cytokines, airway epithelial cells respond by raising steady-state levels of HBD mRNA[16] Further study[7] revealed that HBD-2 could up-regulate the expression of chemoattractants, including interleukin-1 (IL-1β), IL-6, IL-8, monocyte chemoattractant protein (MCP)-1, MCP-2, growth-related oncogene (GRO), and macrophage-derived chemokine (MDC), which are crucial in the development and expansion of the early response to bacterial pathogens. This may indicate that HBD-2 acts to influence cells within the immediate microenvironment to their site of induction, to recruit and activate other cells, perpetuating the response to pathogenic microorganisms in the host defense mechanism. IL-8 attraction of neutrophils would lead to further α-defensin production, maintaining the antimicrobial environment, which is broadened by the action of certain chemokines. In this context, it is worth noting that HBD-2 up-regulated certain pro-inflammatory cytokines, which in turn are able to promote HBD-2 expression in epithelial cells, thus triggering an amplification circuit.

In our study, the decreased plasma HBD-2 levels on admission were associated with the higher possibility of the need for invasive mechanical ventilation and development of new complications, and 30-day mortality showed a trend in association with decreased plasma HBD-2 levels. Lower HBD-2 < 12.5 mg/L was not only a potential cutoff point for the requirement of invasive ventilation and the development of complicated pneumonia, but also increased the possibility of higher mortality in the CAP sub-

jects. Further "dose response" using quartiles of HBD-2 levels showed that the plasma HBD-2 levels on admission were associated significantly with unadjusted parapneumonic pleural effusion, empyema, and other complication. In addition, plasma HBD-2 levels correlated with CURB-65 score, indicating indirectly its association with the severity of CAP. However, there was no difference in ICU stay or mechanical ventilation time in the subjects' stratification using quartiles of HBD levels; thus, the clinical implications of HBD-2 levels should be further studied in patients with severe CAP.

Smoking was not shown as an independent prognostic factor in multivariable analysis in our study, and the association between cigarette smoking and adverse outcomes remains uncertain. This may be due to the suppression of HBD-2 expression in the airway epithelium by cigarette smoking, and resulted in less potent response from the lung. A similar report revealed that a significantly decreased concentration of HBD-2 was found in pharyngeal flushing fluid from current smokers and former smokers, compared with never smokers.[22] Generally, however, the plasma HBD-2 levels measured on admission correlated with total adverse outcomes in subjects with CAP, and the lower plasma HBD-2 levels on admission might predict worse short-term prognosis.

Our results seem to conflict with the recent study[23] in which Leow and colleagues reported the lack of association of HBD-2 and CAP in a small sample size study. But the primary end point of their study was 30-day mortality, while that of our study focused on adverse outcomes instead of mortality only. Even though the sample size of our study was slightly larger than that of their study, we had to admit that our small sample size limited the complexity of the analyses and the extent to which we could adjust for multiple potential confounding factors.

This study has several limitations that should be acknowledged. First, the subjects with CAP in our study were not stratified by pathogen, as pathogen could not be identified in many subjects with CAP. Therefore the relationship between elevation of HBD-2 and pathogen was not clarified. Second, the potential influence of antibiotics was not precluded. Use of antibiotics prior to admission was not excluded because most subjects would have received antibiotics, either self-prescribed or given as empiric antibiotics as standard of care, which might not have been recorded or recalled. Third, the sample size of our study is not large enough; thus, only preliminary data could be provided, and the area under the curve and Youden index were somewhat lower. Further multicenter study may be needed to provide better results, which is suggestive of combination of HBD-2 with other markers to assess the prognosis of CAP. Confounding factors, including various types of comorbidities, were not removed completely, as CAP occurred more often in subjects with multiple comorbidities. Additionally, we were primarily interested in the association of the HBD-2 levels and prognosis of subjects with CAP, and no data on secondary infections were available. More detailed clinical data, including secondary infections of CAP, will be included in future studies. Nevertheless, we did exclude patients with iatrogenic or acquired immunosuppression.

Conclusions

In conclusion, plasma HBD-2 levels on admission, CURB-65 score, and age correlated with the short-term prognosis in subjects with CAP. Plasma HBD-2 level is an independent risk factor for the prognosis in CAP subjects. The lower plasma HBD-2 level on admission indicates worse prognosis. Plasma HBD-2 levels may become another useful tool for prognostic stratification and management of subjects with CAP if validated further in multicenter research.

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