

Risk factors for mortality in patients with *Acinetobacter baumannii* bloodstream infection with genotypic species identification

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Background and Purpose: *Acinetobacter baumannii* is an increasingly common nosocomial infection with a high mortality rate. Identification of predictor factors of mortality from *A. baumannii* infection is important for the implementation of therapeutic management for patients with higher risk. However, many studies have reported data for *Acinetobacter calcoaceticus*-*A. baumannii* complex, which might lead to an uncertainty of results. In this study, we aimed to identify the predictive factors for mortality of patients infected with true *A. baumannii* that had been precisely identified by genotypic methodology.

Methods: Sixty seven patients with documented *A. baumannii* bacteremia were identified from a medical center in northern Taiwan during the period between February 1998 and February 2001. The patients' medical records were retrospectively reviewed.

Results: The risk factors associated with mortality in patients with *A. baumannii* bacteremia were underlying disease with malignancy, end-stage renal disease, and inappropriate antibiotic therapy. Laboratory variables, such as creatinine level, were also associated with poor prognosis by multivariate analysis.

Conclusions: Increased serum creatinine level, malignancy and inappropriate therapy within 3 days were related to increased mortality in patients with *A. baumannii* bloodstream infection. Physicians should be aware of patients with poor prognostic factors and initiate prompt strategies, including appropriate antimicrobial therapy, in order to reduce mortality.

Key words: *Acinetobacter baumannii*; Bacteremia; Mortality; Risk factors; Sequence analysis, DNA

Introduction

Acinetobacter baumannii, an aerobic, non-fermentative Gram-negative coccobacillus, is a well-known cause of hospital-acquired infection. The organism can cause a variety of infections, including pneumonia, urinary tract infection, soft tissue infection, meningitis, and bloodstream infection [1-4]. Infections associated with *A. baumannii* typically have high mortality rates, especially in patients with serious underlying diseases.

A. baumannii bacteremia increases morbidity and prolongs hospital stay [5]. Multidrug-resistant strains of *A. baumannii* have been observed to develop rapidly, via the acquisition and transfer of antibiotic resistance genes on plasmids, transposons and integrons, and pose a great therapeutic challenge [6-10]. Prognostic factors for mortality due to *A. baumannii* bacteremia have been studied previously [1,4,11-14]. However, such data have often been retrieved from patients infected with *Acinetobacter calcoaceticus*-*A. baumannii* (Ac-Ab) complex, which might lead to an uncertainty in the identification of specific risk factors [1,4,11-14]. The aim of this study was to identify predictive factors of mortality of true *A. baumannii* bloodstream infections.

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Methods

Bacterial isolates, identification of *Acinetobacter baumannii*, and susceptibility testing

Bacteremic isolates of Ac-Ab complex were collected from Taipei Veterans General Hospital, Taipei, Taiwan, during the period February 1998 to February 2001. The blood specimens were processed with the BACTEC™ system (NR 660; Becton Dickinson, Sparks, MD, USA). The bacteria were phenotypically identified as Ac-Ab complex by use of the API ID 32 GN kit (bioMérieux, Marcy l'Etoile, France). Genomic species identification of *A. baumannii* was carried out by a multiplex-polymerase chain reaction (PCR) method [15]. This PCR method includes 2 pairs of primers. The first pair of primers, P-Ab-ITSF: 5'CATTATCACGGTAATTAGTG3' and P-Ab-ITSB: 5'AGAGCACTGTGCACTTAAG3', specifically amplified an internal fragment of 208 bp from the ITS region of *A. baumannii*. A second pair of primers, P-rA1: 5'CCTGAATCTTCTGGTAAAAC3' and P-rA2: 5'GTTTCTGGGCTGCCAAACATTAC3', which target a highly conserved region of the *recA* gene (about 425 bp) of *Acinetobacter* spp., was included as a reaction control. The PCR reaction mixture was first subjected to 94°C for 5 min, and then 30 cycles of amplification were performed with template DNA denaturing at 94°C for 30 sec, primer annealing at 55°C for 30 sec, and primer extension at 72°C for 30 sec. The final extension step was 7 min at 72°C. Amplified products were electrophoresed in a Tris-acetate-ethylenediamine tetra-acetic acid (TAE)-buffered 1.5% agarose gel. Isolates that gave 2 fragments were identified as *A. baumannii* and those that gave only the fragment corresponding to *recA* were classified as species belonging to non-*baumannii* Ac-Ab complex.

Antimicrobial susceptibility testing was performed using the agar dilution method, according to the Clinical Laboratory Standards Institute (CLSI; formerly National Committee for Clinical Laboratory Standards [NCCLS]) guidelines [16].

Definitions

The medical records of the infected patients were retrospectively reviewed for information on risk factors and underlying conditions. Retrieved data included demographic characteristics, underlying diseases, invasive procedures, laboratory examinations, and antimicrobial treatments.

An *A. baumannii* bacteremia episode was defined as isolation of one or more isolates of *A. baumannii* from blood in a patient on 1 or more occasions [1]. The infection was considered hospital-acquired if it developed 48 hours or more after admission.

Underlying diseases were divided into several categories, including malignancy, previous cerebral vascular accident, type 2 diabetes mellitus, hypertension, end-stage renal disease, congestive heart failure, chronic obstructive pulmonary disease, and immunosuppressive status. Immunosuppressive status was defined as patients having one of the following: solid organ or stem cell transplantation, human immunodeficiency virus infection, or treatment with cytotoxic chemotherapy within the previous 6 weeks or more than 2 doses of steroids or other immunosuppressive agents within 2 weeks prior to the first episode of *A. baumannii* bacteremia [1]. End-stage renal disease was defined as creatinine clearance rate <5 mL/min that required hemodialysis. Central venous catheterization and ventilator usage were considered risk factors if performed or existing within 48 hours prior to the first *A. baumannii* bacteremia episode. Antimicrobial therapy was considered as appropriate if *A. baumannii* isolated from blood was susceptible in vitro to at least one of the empirical antibiotics used. Death of a patient was considered directly related to the bacteremia if it happened in the phase of active infection without evidence of any other attributable cause [17].

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) for Windows (Version 13; SPSS, Chicago, IL, USA) is the software that was applied for all data analysis. Chi-squared test with Yates' correction or Fisher's exact test was used to compare categorical differences. Mann-Whitney *U* test was used to analyze continuous variables. A multivariate analysis with logistic regression was performed to identify risk factors independently associated with mortality from *A. baumannii* bacteremia. A *p* value of 0.1 was the limit for entering or removing variables. A *p* value <0.05 was considered statistically significant.

Results

During the study period, a total of 142 patients (54 males) suffered from bacteremia due to Ac-Ab complex. Among these patients, 67 were infected by genomic

species *A. baumannii*. The age of the patients ranged from 17 to 94 years (mean, 65.41 years). There were 37 patients from intensive care units, 26 patients from ordinary wards, and 4 from emergency rooms. The mean hospital stay was 68.5 days (range, 2-312 days). The major underlying diseases and conditions included

chronic obstructive pulmonary disease (41.8% of patients), old cerebral vascular accident (35.8%), congestive heart failure (35.8%), malignancy (28.4%), immunosuppressive status (22.4%), end-stage renal disease (19.4%), neutropenia (14.9%) and abdominal surgery within 1 month (10.4%).

Table 1. Demographic and risk factors associated with mortality in patients with *Acinetobacter baumannii* bloodstream infection

Variable	Total	Survival group No. (%)	Mortality group No. (%)	<i>p</i>
Total	67	44 (65.7)	23 (34.3)	
Gender				0.981
Male	54	36 (66.7)	18 (33.3)	
Female	13	8 (61.5)	5 (38.5)	
Ward category				0.494
Intensive care unit	37	23 (62.2)	14 (37.8)	
Ordinary ward	26	19 (73.1)	7 (26.9)	
Emergency room ^a	4	2 (50.0)	2 (50.0)	
Nosocomial infection				0.510
No	3	3 (100.0)	0 (0.0)	
Yes	64	41 (64.1)	23 (35.9)	
Old cerebral vascular accident				0.230
No	43	24 (55.8)	19 (44.2)	
Yes	24	20 (83.3)	4 (16.7)	
Congestive heart failure				0.506
No	43	27 (62.8)	16 (37.2)	
Yes	24	17 (70.8)	7 (29.2)	
Chronic obstructive pulmonary disease				0.213
No	39	28 (71.8)	11 (28.2)	
Yes	28	16 (57.1)	12 (42.9)	
End-stage renal disease				0.009
No	54	40 (74.1)	14 (25.9)	
Yes	13	4 (30.8)	9 (69.2)	
Immunosuppressive status				0.000
No	52	40 (76.8)	12 (23.1)	
Yes	15	4 (26.7)	11 (73.3)	
Neutropenia				0.000
No	57	43 (75.4)	14 (24.6)	
Yes	10	1 (10.0)	9 (90.0)	
Malignancy				0.000
No	48	38 (79.2)	10 (20.8)	
Yes	19	6 (31.6)	13 (68.4)	
Abdominal surgery within 1 month				0.356
No	60	41 (68.3)	19 (31.7)	
Yes	7	3 (42.9)	4 (57.1)	
Central venous catheter insertion				0.133
No	16	13 (81.3)	3 (18.8)	
Yes	51	31 (60.8)	20 (39.2)	
Ventilator use				0.202
No	13	11 (84.6)	2 (15.4)	
Yes	54	33 (61.1)	21 (38.9)	
Appropriate antibiotics within 3 days				0.036
No	53	31 (58.5)	22 (41.5)	
Yes	14	13 (92.9)	1 (7.1)	

^aIncluding observation rooms.

Table 2. Laboratory data for survival and mortality groups in patients with *Acinetobacter baumannii* bloodstream infection

Risk factor	Survival group				Mortality group				p
	No.	Mean ± SD	Median	Range	No.	Mean ± SD	Median	Range	
WBC (/mm ³)	42	13,006 ± 6112	12,700	1670-29,400	23	10,718 ± 11,214	10,718	40-41,600	0.078
Hemoglobin (g/dL)	42	10.3 ± 1.77	9.75	7-15	23	9.15 ± 2.32	9.15	6-16	0.025
Platelets (cells/mm ³)	42	201,821 ± 138,804	162,000	34,500-753,000	23	66,000 ± 67,349	66,000	4000-226,000	0.000
CRP (mg/dL)	26	9.17 ± 8.21	7.33	1-38	15	18.18 ± 11.398	18.18	7-44	0.002
Albumin (g/dL)	32	3.01 ± 0.48	3.05	2-4	18	2.76 ± 0.362	2.76	2-3	0.041
BUN (mg/dL)	42	31.33 ± 23.04	23	6-99	23	75.83 ± 54.128	75.83	15-202	0.000
Creatinine (mg/dL)	42	1.22 ± 0.687	1.1	0-3	23	2.57 ± 1.364	2.57	0-6	0.000
Ventilator use (days)	33	50.33 ± 89.20	19	1-393	21	40.14 ± 50.44	40.14	2-236	0.097

Abbreviations: SD = standard deviation; WBC = white blood cell; CRP = C-reactive protein; BUN = blood urea nitrogen

Patients were divided into those who died of the infection (mortality group) and those who did not (survival group). The comparative demographic data and underlying diseases are shown in Table 1. Univariate analysis revealed that predictive factors for mortality included end-stage renal disease (mortality vs survival group, 69.2% vs 30.8%, $p=0.009$), immunosuppressive status (73.3% vs 26.7%, $p=0.000$), malignancy (68.4% vs 31.6%, $p=0.000$) and neutropenia (90.0% vs 10.0%, $p=0.000$).

Laboratory data significantly associated with mortality included lower hemoglobin level (mortality vs survival group, 9.15 g/dL vs 10.30 g/dL, $p=0.025$), platelet count (66,000/mm³ vs 201,821/mm³, $p=0.000$), albumin level (2.76 g/dL vs 3.01 g/dL, $p=0.041$), C-reactive protein (18.18 mg/dL vs 9.17 mg/dL, $p=0.002$), blood urea nitrogen (75.83 mg/dL vs 31.33 mg/dL, $p=0.000$) and creatinine level (2.57 mg/dL vs 1.22 mg/dL, $p=0.000$)

[Table 2]. Inappropriate antibiotic usage within 3 days was also related to higher mortality (7.1% vs 41.5%, $p=0.036$).

The antimicrobial susceptibilities of isolates in the survival and mortality groups were mostly comparable (Table 3). However, a higher proportion of isolates in the mortality group were resistant to imipenem ($p=0.074$).

Multivariate analysis by logistic regression identified end-stage renal disease, malignancy, inappropriate antibiotic therapy within 3 days, and higher creatinine level as the independent factors associated with mortality (Table 4).

Discussion

A. baumannii has been documented as an important nosocomial pathogen with high mortality risk

Table 3. Susceptibility of *Acinetobacter baumannii* isolates

Antimicrobial agent	Non-susceptible isolates (n = 67)		p
	Survival group (n = 44)	Mortality group (n = 23)	
Ampicillin-sulbactam	38 (86.3)	21 (91.3)	0.554
Ticarcillin-clavulanic acid	42 (95.5)	21 (91.3)	0.496
Piperacillin-tazobactam	44 (100.0)	21 (91.3)	ND
Ceftriaxone	44 (100.0)	22 (95.7)	ND
Cefepime	42 (95.5)	21 (91.3)	0.496
Imipenem	3 (6.8)	5 (21.7)	0.074
Gentamicin	42 (95.5)	21 (91.3)	0.496
Tobramycin	41 (93.0)	21 (91.3)	0.781
Amikacin	44 (100.0)	21 (91.3)	ND
Ciprofloxacin	42 (95.5)	23 (100.0)	0.299
Trimethoprim-sulfamethoxazole	43 (97.7)	22 (95.7)	0.636
Aztreonam	44 (100.0)	21 (91.3)	ND
Levofloxacin	36 (81.8)	21 (91.3)	0.301

Abbreviation: ND = not done

Table 4. Risk factors of attributable mortality for *Acinetobacter baumannii* bloodstream infection: multivariate analyses of relative risk of death

Risk factor	OR (95% CI)	<i>p</i>
Underlying condition		
End-stage renal disease	59.82 (5.384-664.882)	0.001
Immunosuppressive status	0.130 (0.004-3.800)	0.236
Neutropenia	47.07 (0.692-3202.682)	0.074
Malignancy	10.84 (1.397-84.144)	0.023
Proper antibiotic use	20.71 (1.317-325.953)	0.031
Laboratory data		
White blood cells (cut-off = 10,000/mm ³)	4.04 (0.253-64.511)	0.323
Hemoglobin (cut-off = 9 g/dL)	0.613 (0.060-6.271)	0.680
Platelet (cut-off = 100,000/mm ³)	8.934 (0.369-216.156)	0.178
C-reactive protein (cut-off = 10 mg/dL)	6.892 (0.488-97.335)	0.153
Albumin (cut-off = 2.85 g/dL)	0.239 (0.005-11.187)	0.465
Blood urea nitrogen (cut-off = 50 mg/dL)	0.253 (0.013-4.914)	0.364
Creatinine (cut-off = 2 mg/dL)	- (1.395-260.881)	0.027

Abbreviations: OR = odds ratio; CI = confidence interval

[18]. However, it is impossible to differentiate this genomic species from other species belonging to Ac-Ab complex, including genomic species 3 and 13 TU (13 sensu Tjernberg and Ursing), by the phenotypic test that is used routinely in the clinical laboratory. Misidentification among the Ac-Ab complex might lead to the wrong clinical impression regarding the pathogenesis of different *Acinetobacter* species [19]. A similar phenomenon can be seen with *Burkholderia cepacia*, which occurs as 9 different genomic species that show morphological similarity, but different clinical features and presentations [20]. Recent reports have focused on prognostic factors derived mainly from Ac-Ab complex, which may not reflect accurately the true prognostic factors of *A. baumannii*. Our data were obtained from patients infected with real *A. baumannii*.

Choi et al demonstrated that higher Acute Physiology and Chronic Health Evaluation (APACHE) II score tended to be associated with higher mortality rate [13]. Although we did not assess the severity of patients' conditions, our patients with immunocompromised states were at increased risk of mortality. The immunocompromised states included end-stage renal disease, immunosuppressive status, and malignancy. A similar result was noted from another study showing a higher percentage of deaths in patients with malignant tumors. Neutropenia was also found to be a risk factor for mortality in *A. baumannii* bacteremia, a finding which may relate to poor immune status [13].

Central venous insertion and mechanical ventilation were not statistically significant predictors of mortality in this study. However, other studies of Ac-Ab complex

suggested that mechanical ventilation is independently associated with poor prognosis [13,14]. The discrepancy in these results might be due to the inclusion of different proportions of *A. baumannii* infection among the studies. Our data showed that higher creatinine level was related to higher mortality, even at levels that had not reached end-stage renal disease.

Interestingly, the rate of imipenem resistance was higher in the mortality group than in the survival group ($p=0.074$). These imipenem-resistant isolates were also resistant to other antibiotics, which resulted in a higher rate of inappropriate therapy in this patient group. Thus, appropriate antibiotic choice is crucial in this clinical situation.

Our study revealed that inappropriate therapy within 3 days of *A. baumannii* bacteremia was associated with a poor prognosis, which had also been noted previously in patients infected with Ac-Ab complex [13]. Nosocomial *A. baumannii* isolates are often multidrug-resistant; rapid identification of this species with prompt initiation of potent antibiotics is needed in order to improve clinical outcomes.

In conclusion, this study showed that increased serum creatinine level (including end-stage renal disease), malignancy and inappropriate therapy within 3 days were related to increased mortality from *A. baumannii* bloodstream infection.

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