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Evaluation of humoral immune response to nosocomial pathogen and functional status in elderly patients with sepsis

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ABSTRACT

The clinical significance of humoral immune response to nosocomial pathogens and functional status in elderly patients with sepsis is not clear. We evaluated the humoral immune to nosocomial pathogens and the effect of functional dependencies on clinical outcomes among elderly patients with sepsis. This study prospectively enrolled patients aged ≥65 years with sepsis from September 2011 to May 2012 at a 2000-bed university hospital. The data including CD4 and CD8 T-cell count, functional status by measuring basic activities of daily living (ADL) and instrumental activities of daily living (IADL) were collected for all patients. In addition, the collected blood samples were analyzed for serum antibody levels against nosocomial pathogens using an ELISA. During the study period, 72 patients (38 males) treated with sepsis were enrolled. The all-cause in-hospital mortality rate was 16.7% (12/72). The mean CD4/CD8 T-cell ratio was significantly lower in nonsurvivors than in survivors (1.08 ± 0.72 vs. 1.93 ± 1.42, $P = 0.003$). Serum antibody titers to *Acinetobacter baumannii*, *Klebsiella pneumonia*, *Stenotrophomonas maltophilia*, and *Enterococcus faecalis* were statistically higher in nonsurvivors than in survivors. On multivariate analysis, the IADL score was independently predictive of mortality in elderly patients with sepsis (odds ratio 1.410, 95% confidence interval 1.007–1.975, $P = 0.046$). These results suggest that IADL scores could be used as predictors to identify elderly patients with a poor prognosis of nosocomial infections.

1. Introduction

Sepsis is a life-threatening immune response to an infection that contributes to nearly 20% of all in-hospital deaths. The incidence of severe sepsis increases with age and septic patients have a mean age of around 65 years. Older adults are more likely to present with attenuated or atypical manifestations of infection than younger adults (Chassagne et al., 1996) and are more likely to develop an infection caused by antimicrobial-resistant bacteria (Diekema, Pfaller, & Jones, 2002). In both situations, there is considerable delay in initiating antimicrobial therapy with activity against the pathogen. Aging is associated with well-described changes in adaptive humoral and cell-mediated immunity (Grubeck-Loebenstein & Wick, 2002), as well as abnormal cytokine responses during episodes of septicaemia (Opal, Girard, & Ely, 2005). The decline of immune function that accompanies aging is prominent among many factors that contribute to the increased susceptibility of elderly patients to sepsis (Opal et al., 2005).

Despite significant advances in knowledge of the microbial characteristics of nosocomial pathogens, including *Acinetobacter baumannii*, many aspects of the host response to nosocomial infections have not been fully studied. A few studies have analyzed relationship between serum IgG antibody against bacteria and the clinical aspects (Sugi et al., 2011; Tyski, Jarecka, Gut, & Hryniewicz, 1991).

Functional impairment, a common occurrence in aging, is generally measured as any difficulty that interferes with or limits function in one or more major life activities, including basic activities of daily living (ADL) and instrumental activities of daily living (IADL) (Katz, Downs, Cash, & Grotz, 1970; Lawton & Brody, 1969). Previous reports have described a relationship between markers such as C-reactive protein (CRP), interleukin-6 (IL-6), and D-dimer and measures of functional impairment in older populations (Cohen, Harris, & Pieper, 2003; Taaffe, Harris, Ferrucci, Rowe, & Seeman, 2000). Ferrucci et al. (1999) suggest that cytokine overproduction and maintenance of the inflammatory state over a

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long period may lead to physical degeneration in the elderly population.

It is unclear how the presence and levels of systemic antibodies against nosocomial pathogens reflect inflammatory disease activity in elderly patients with sepsis. In addition, the influence of functional impairment has not been clearly assessed in elderly patients with sepsis, a population at high risk for poor outcomes.

The aims of the present study were to evaluate humoral immune status to nosocomial pathogens and to investigate the effect of functional dependency on clinical outcomes among elderly patients with sepsis.

2. Patients and methods

2.1. Study design and population

The study was carried out at Severance Hospital, a 2000-bed university-affiliated tertiary care referral hospital in Seoul, Republic of Korea. This was a prospective study of patients treated for sepsis. The study took place from September 1, 2011 to May 31, 2012 and the Institutional Review Board of Severance Hospital approved the protocol. All participants provided written informed consent.

Patients with sepsis were assessed for inclusion, which required that patients were older than 65 years of age with confirmed or presumed infection. Analysis was based on 30-day mortality except cancer related mortality.

2.2. Data collection

The following data were collected: age, gender, co-morbid conditions, duration of hospital stay, levels of ESR and CRP, source of infection, CD4 and CD8 T-cell count, and routine blood tests.

The functional status of individuals was assessed in terms of their ability to perform important ADL and IADL without help. ADL measurement consisted of seven items, and was used to measure the ability to perform activities such as bathing, walking, transfer, dressing, personal grooming, eating, and using the toilet (Katz et al., 1970). IADL measurement consisted of 4 items and was used to measure ability to perform activities such as preparing meals, managing money, managing medications, and using a telephone (Lawton & Brody, 1969). All the ADL and IADL items were coded as "1" if the participant was independent, "2" if the participant needed some assistance, or "3" if the participant was completely dependent in performing the activity. The ADL and IADL scale rages were 7–21 and 4–12, respectively, where a higher number indicates higher impairment for the specific task.

2.3. Bacterial strains

Seven nosocomial pathogen strains were used for this study: methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33591, *Acinetobacter baumannii* (ABA) ATCC 19606, *Klebsiella pneumonia* (KPN) ATCC 13883, *Pseudomonas aeruginosa* (PAE) PAO1, *Stenotrophomonas maltophilia* (SMP) ATCC 13637, *Enterococcus faecalis* (EFA) ATCC 29212, and *Enterobacter aerogenes* (EA) KCTC 2190.

2.4. Antigen preparation and serum antibody assay

Blood samples were collected and specimens were immediately centrifuged and stored at −70 °C until tested. Serum antibody levels were determined using an ELISA. The amount of serum IgG that bound to each pathogenic bacteria antigen was measured by a modified method as described previously (Sugi et al., 2011). Briefly, bacteria grown in Luria-broth (LB, 10 g tryptone, 10 g NaCl, 5 g yeast extract per liter) were re-inoculated in microtiter plate wells (Nunc, Maxisorp). Microtiter plate wells were coated with 100 μL aliquots of bacteria in 0.1 M carbonate buffer (pH 9.6) at a concentration of 1 μg/mL. To allow quantification of antibody titer, 100 μL aliquots of known concentrations of IgG prepared from purified human IgG (Zymed) were also transferred to the plate. Negative control wells were prepared for each serum sample by adding carbonate buffer to only the appropriate wells. After overnight incubation at 4 °C, the plates were washed (×3) in phosphate-buffered saline (PBS)–Tween and blocked with 1% BSA for 1 h at room temperature. One hundred-microliter aliquots of diluted serum were then added to the appropriate wells, and the plates were incubated at room temperature for 2 h. After further washes (×3) in PBS-T, plates were incubated for 1 h at room temperature with diluted rabbit anti-human IgG-specific horseradish peroxidase-labeled monoclonal antibody (Dako). After further washes (×3) in PBS-T, color development was achieved by adding 150 μL of 2.5 mM tolidine (Eastman Kodak, Rochester, NY) in 100 mM phosphate citrate buffer (pH 3.5) containing 0.025 mM ethylenediaminetetraacetic acid and activated with 3% H_2O_2. The resulting reaction was stopped after 10 min by adding 50 μL of 1 M HCL. Plates were then read at 450 nm and 655 nm.

2.5. Statistical analysis

All variables are expressed as the mean ± standard deviation (SD), unless otherwise indicated. Associations among dichotomous variables were assessed using contingency tables to determine Pearson's χ^2 and linear χ^2 functions. Continuous variables were compared by the Student's t test and Mann–Whitney U test. Multivariate analysis was performed using a logistic regression model to estimate the odds ratio (OR) of dying along with 95% confidence intervals (CIs). CIs were calculated using the iterative profile likelihood method. The model was chosen using stepwise regression, starting from the null model, to choose parameters. Statistical analyses were performed using the Statistics Package for Social Science (SPSS 18.0 for Windows; SPSS Inc., Chicago, IL, USA). *P*-Values less than 0.05 were considered statistically significant.

3. Results

From September 2011 to May 2012, 72 patients (38 males) with sepsis were enrolled in the study. Clinical characteristics of the patients are shown in Table 1. The mean age of patients was 78.4 ± 7.9 years, and the all-cause in-hospital mortality rate was

Table 1
Clinical characteristics of patients.

Characteristic	Survivors (n = 60)	Nonsurvivors (n = 12)	*P*-Value
Age (years)	78.5 ± 7.7	77.8 ± 9.7	0.803[a]
Male, n (%)	30 (50.0)	8 (66.7)	0.291[b]
Underlying diseases (yes), n (%)			
Solid cancer	10 (16.7)	8 (66.7)	0.001
Hematologic malignancy	1 (1.7)	0 (0.0)	1.000
Primary hypertension	48 (80.0)	11 (91.7)	0.681
Cardiovascular disease	24 (40.0)	4 (33.3)	0.755
Chronic heart failure	12 (20.0)	1 (8.3)	0.681
Chronic renal disease	14 (23.3)	5(41.7)	0.280
Chronic liver disease	2 (3.3)	1 (8.3)	0.426
Chronic lung disease	4 (6.7)	2 (16.7)	0.260
Old TB	3 (5.0)	3 (25.0)	0.504
DM	22 (36.7)	7 (58.3)	0.204

SD, standard deviation; TB, tuberculosis; DM, diabetes mellitus.
Data are expressed as mean ± SD or number (percent).
[a] Student's t test.
[b] Pearson's χ.