SHOCK, Vol. 60, No. 3, pp. 354–361, 2023

OPEN

UTILITY OF STREM-1 BIOMARKER AND *HCP* GENE FOR IDENTIFICATION OF *ACINETOBACTER BAUMANNII* COLONIZATION AND INFECTION IN LUNG

Xiaolei Wang,*† Jiahui Jiang,*‡ Chenxing Wei,† Wenjie Yang,*† Jian Chen,† Xueyan Dong,*† Haitong Wan,* and Daojun Yu*†

**The Fourth School of Clinical Medicine, Zhejiang Chinese Medical University, Hangzhou, China; †Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine, Hangzhou, China; and ‡Cancer Center, Key Laboratory of Tumor Molecular Diagnosis and Individualized Medicine of Zhejiang Province, Zhejiang Provincial People's Hospital (Affiliated People's Hospital, Hangzhou Medical College), Hangzhou, Zhejiang, China*

Received 7 May 2023; first review completed 5 Jun 2023; accepted in final form 27 Jun 2023

ABSTRACT—**Objective:** Respiratory infections or colonization of *Acinetobacter baumannii* (Ab) are common in clinical practice but are treated differently. Early identification of Ab infection and colonization reduces the risk of antibiotic mismatch but objective laboratory indicators to distinguish between bacterial infections and colonization are lacking. To distinguish infection and colonization of Ab, we tested the role of two biomarkers, triggering receptor expressed on myeloid cells-1 (TREM-1) and hemolysin coregulated protein. **Methods:** A total of 96 inpatients with Ab were divided into infection and colonization groups. Blood samples were collected on days 1, 2, 3, 5, 8, and 10 and daily maximum body temperature was recorded. Polymerase Chain Reaction and Reverse Transcription Polymerase Chain Reaction were used to detect the presence and expression levels of the *hcp* gene in Ab clinical isolates. **Results**: sTREM-1 and procalcitonin (PCT) levels on days 1 to 10 and neutrophil classification (N%) on days 1 to 3 were different ($P < 0.05$) in the infection group and colonization group. Receiver operating characteristic (ROC) curves showed significant differences in N% and sTREM-1 on days 2 and 3 ($P < 0.01$). sTREM-1 had the highest AUC^{ROC} on days 1, 2, and 3 of all the markers. On day 1, the ROC curve of "WBC&N%&PCT&sTREM-1" was statistically different from individual indices (white blood cell count, N%, and PCT; $P < 0.05$) and was equal to the ROC curve of sTREM-1 ($P > 0.05$). Thirty five of 96 patients were classified as infection group and 61 as colonization group with *hcp* gene detection rates of 71.43% (25/35) and 31.15% (19/61), respectively. No differences in *hcp* gene presence and transcript levels were found between two groups ($P > 0.05$). **Conclusions:** Dynamic monitoring of sTREM-1 and PCT is valuable in identifying Ab infection and colonization. sTREM-1 can be improved by combination with multiple biomarkers in the early stage for identification of infection and colonization. The *hcp* gene was more likely to be present in the infection cohort.

KEYWORDS—*Acinetobacter baumannii* (Ab); soluble triggering receptor expressed on myeloid cells-1 (sTREM-1); procalcitonin (PCT); infection; colonization; hemolysin coregulated protein (Hcp)

INTRODUCTION

Acinetobacter baumannii (Ab) is a gram-negative bacterium responsible for bacteremia, pneumonia, meningitis, and urinary tract infections in patients with impaired immune function (1). *Acinetobacter baumannii* is carried by up to 25% of healthy people with normal immune systems and accounts for approximately 20% of intensive care unit (ICU) infections worldwide (2). Lung Ab colonization, which occurs frequently insusceptible populations, is a prerequisite for infection (3). Early distinction of Ab infection and colonization reduces the risk of antibiotic misprescription, development of drug-resistant bacteria, and prolonged hospital stays. Mistaking infection for colonization may delay treatment, worsening a patient's condition with potentially fatal results. Therefore, early identification of *A. baumannii* infection or colonization is of great significance.

However, clinical experimental data and diagnosis and treatment guidelines for distinction of bacterial infection and colonization are lacking. Clinicians identify pulmonary Ab infection or colonization based on the results of bacterial culture, white blood cell count (WBC) and neutrophil classification (N%), C-reactive protein (CRP), procalcitonin (PCT), and other plasma biomarker detection results combined with time consuming and subjective imaging and symptoms analysis (4). This method not only wastes time but also depends on the subjective judgment of doctors. The accuracy rate of identification of lung Ab infection or colonization remains nonideal.

Triggering receptor expressed on myeloid cells-1 (TREM-1) is a transmembrane glycoprotein selectively expressed by intrinsic immune cells such as neutrophils, mature monocytes, macrophages, and platelets. It is involved in the pathophysiology of acute and chronic inflammatory diseases with different etiologies in which it triggers inflammation, regulates production of inflammatory factors, and amplifies inflammatory signals (5). The expression of TREM-1 is upregulated during infection and the soluble form of TREM-1, sTREM-1, reflects inflammatory status (6). The level of sTREM-1 correlated with infection severity, including hospital acquired pneumonia (HAP) caused by Ab and was barely detectable in patients with nonmicrobial inflammation (7). Feng et al. (8) demonstrated a positive correlation between serum sTREM-1 and sputum bacterial load. Hence, sTREM-1

Address reprint requests to Haitong Wan, Zhejiang Chinese Medical University, No. 548 Binwen Rd., Hangzhou, Zhejiang, China, 310053, E-mail:wanhaitong@zjtcm.net; Co-correspondence: Daojun Yu, Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine, No. 261 huansha Rd., Hangzhou, Zhejiang, 310006. E-mail:yudaojun98@zju.edu.cn

XW, JJ, CW, and WY contributed equally to this work.

This work was supported by the National Natural Science Foundation of China (grant number 81930111), the Natural Science Foundation of Zhejiang Province (grant number LZ22H190002), the Health & Medical Sci-Tech Project of Hangzhou Municipal Health Commission (Z2021005), the Science and Technology Project of Hangzhou Municipal (grant number 202204A001), and the Health & Medical Sci-Tech Project of Health Commission of Zhejiang Province (grant number 2020KY206, 2022PY016).

The authors report no conflict of interests.

DOI: 10.1097/SHK.0000000000002175

in serum may have significance in identifying the infection or colonization of *A. baumannii* (2).

Currently, WBC, CRP, and PCT are used for clinical distinction between infection and colonization (4). White blood cell count increases in response to infection and inflammation but is also affected by physiological factors, resulting in poor specificity. C-reactive protein is a liver-derived positive acute phase protein, which increases in serum in response to infection, tumors, or autoimmune diseases, giving it high sensitivity but low specificity (9). Procalcitonin is a specific inflammatory infection marker related to the severity of bacterial infection but affected by immune status, microorganism type, and noninfectious diseases, such as cancer (10). Therefore, WBC, CRP, and PCT are insufficient for clinical differentiation of infection or colonization.

The Ab type VI protein secretion system (T6SS) has been recently identified as increasing bacteria-host competition, enhancing bacterial invasiveness, adhesion, and viability with in macrophages (11,12). Hemolysin coregulated protein (Hcp) is a structural and effector component of T6SS and a marker of functional T6SS (11,13,14). Hemolysin coregulated protein secretion into culture medium has been shown to reflect Ab and human respiratory epithelial cell interactions. The *hcp* gene (*hcp* $^{+}$) has been detected in approximately 31.5% of clinical Ab isolates with variable expression rates (11,14,15). Thus, colonizing and infecting strains of Ab in clinical isolates may show differences in *hcp* gene presence and/or expression levels.

In summary, the sensitivity or specificity of a single biomarker index is insufficient for the differential diagnosis and prognosis judgment of lung infection/colonization. This study investigated the application value of single and combined application of various biomarkers in identifying pulmonary *A. baumannii* infection or colonization by jointly detecting the level of WBC, N%, CRP, PCT, and sTREM-1 and other infection markers in blood, as well as the level and expression of *hcp* gene of clinical Ab isolates. The aims were to aid clinical diagnosis and inform treatment decisions.

METHOD

Subjects

A total of 96 patients admitted to Hangzhou First People's Hospital between November 2019 and November 2021 and from whom Ab strains were isolated from lung secretion specimens were enrolled. Fifty-three patients were excluded because of incomplete blood specimens and the remaining 43 patients were divided into the Ab infection (n = 25) and Ab colonization (n = 18) groups by two clinicians independent of the study. Twenty inpatients without infection were selected as a control group.

Grouping criteria and ethics

Patients were retrospectively grouped based on Clinical Pulmonary Infection Scores and the criteria of US Centers for Disease Control and Prevention (16). *Acinetobacter baumannii* colonization in lung was defined as isolation of Ab from lung specimens of patients who did not meet the above HAP criteria. Duplicate strains from the same patient were excluded. Exclusion criteria were as follows: (1) age <18 years; (2) presence of coinfections; (3) severe impairment of liver function; (4) weakened immune system and long-term treatment with hormones or immunosuppressive drugs; (5) presence of hematological disease or malignant tumor, especially lung cancer; and (6) treatment by hemodialysis, blood transfusion or radiotherapy/chemotherapy during hospitalization.

Ethical approval was granted by the ethics committee of Hangzhou First People's Hospital ([2019] KYYLS No. (020)-01) and the need for informed consent was waived.

Patient information, including sex, age, Charlson comorbidity index (CCI) score, number of days in the ICU before Ab detection, number of days of serial detection of Ab, and presence or absence of tracheotomy/intubation, was collated.

Acinetobacter baumannii isolates were preserved on the day of isolation (day 1) and venous blood collected on days 1, 2, 3, 5, 8, and 10. All specimens were stored at −80°C. White blood cell count and CRP were tested immediately. Maximum daily body temperature (*T*, °C) was recorded on days 1, 2, 3, 5, 8, and 10.

Detection of biomarkers

White blood cell count and neutrophil count

The patient's venous blood (EDTA-K2 anticoagulant) was collected, WBC and neutrophil count were measured twice for each sample by the SYSMEX automated modular hematology analyzer with associated reagents (SYSMEX 800i; Sysmex Corporation, Kobe, Japan), and a mean was generated. The biological reference range was 3.5 to 9.5 $\times$ 10^9/L for WBC and 1.8 to 6.3 $\times$ 10^9/L for neutrophil count.

C-reactive protein

The patient's venous blood (EDTA-K2 anticoagulant) was collected and CRP was measured twice for each sample by rate nephelometry using the Mindray automated hematology analyzer with associated reagents (Shenzhen Mindray Corporation, Shenzhen, China) and a mean was generated. The reference range for CRP was 0.2 to 320 mg/L.

Procalcitonin

The venous plasma of patients (heparin lithium anticoagulation) was collected and PCT was measured twice for each sample by luminescence immunoassay using Xiamen Wantai Caris-200 luminescence analyzer with associated reagents (Xiamen Innodx Biotechnology Co, Ltd, Xiamen, China) and a mean was generated. The PCT reference range was 0.02 to 50 ng/mL.

Soluble triggering receptor expressed on myeloid cells-1

The venous plasma of patients (heparin lithium anticoagulation) was collected and sTREM-1 was measured twice for each sample by an enzyme-linked immunosorbent assay using a TREM-1 ELISA Kit (R&D Systems, Minneapolis, MN) according to the operation procedure of the kit. The sTREM-1 reference range was 31.25 to 2,000 pg/mL.

Detection of Ab hcp gene

The preserved Ab isolates were seeded onto Columbia blood agar medium at 37°C for 18 h and transferred to LB broth and shaken at 37°C for 18 h. Bacterial DNA was extracted for quantitative Reverse Transcription Polymerase Chain Reaction and genomic DNA was removed for *hcp* gene expression detection by one-step Transcription Polymerase Chain Reaction. Relative *hcp* transcript levels were calculated using the $2^{-\triangle\triangle ct}$ method and transcript levels of Ab ATCC 17978 used as the control strain.

TABLE 1. **Comparison of basic patient information**

Basic information	Colonization group (n = 18)	Infection group (n = 25)	Control group (n = 20)	Value	*P* value
Sex, male/female	12/6	15/10	12/8	$\chi^2 = 0.292$	0.896
Age, y	74.0 (66.5–79.5)	73.0 (66.0–80.5)	66.5 (60.5–73.8)	$H = 3.428$	0.180
CCI	5.55 (5.0–6.75)	6.0 (4.0–7.0)	5.0 (4.3–7.0)	$H = 0.260$	0.878
Days in ICU before Ab culture positive	8 (4.25–14.75)	10 (5.00–17.00)	—	$z = -0.851$	0.395
Days of continuous Ab culture positive	3.5 (2.0–5.0)	10 (8.0–12.0)	—	$z = -5.46$	0.001
Stay in ICU or not, yes/no	11/7	22/3	14/6	H = 4.253	0.119
Use of antibiotics before admission, with/without	5/13	8/17	7/13	$H = 0.226$	0.893
Whether tracheotomy/intubation was performed, with/without	8/10	17/8	5/15	$H = 8.206$	0.017
Semiquantitative culture results of Ab, small/medium/large amount	3/6/9	3/11/11	—	$z = -0.162$	0.872

Statistical analysis

All data were tested for normality and normally distributed data are expressed as mean ± standard deviation with differences between two independent samples tested by *t* test (2). Nonnormally distributed data are expressed as median (quartiles) (M [QL–QU]) and were tested using Mann-Whitney *U* test (17). The χ^2 test was applied to the count data and ranked data and the corrected χ^2 test was performed when the theoretical number of cells was $1 < T < 5$. receiver operating characteristic (ROC) curves (17) and logistic regression were applied to calculate the AUC^{ROC} (area under the ROC curve), cutoff value, sensitivity/specificity, 95% confidence interval (95% CI), positive likelihood ratio/negative likelihood ratio, and positive predictive value/negative predictive value for evaluation of single and combined indices for identification of Ab colonization and infection. *Z* test was applied for comparison between ROC curves (6). $P < 0.05$ indicated that the difference was statistically significant (2) and SPSS (IBM SPSS Statistics 22.0) was used for data analyses (18).

RESULTS

Study population characteristics

Forty-three patients with complete data were divided into infection (n = 25) and colonization (n = 18) groups and 20 patients without infectious disease enrolled as controls (Table 1). The presence or absence of tracheotomy/intubation and the number of days of consecutive Ab detection in lung specimens were

TABLE 2. **Changes in dynamic indexes of *A. baumannii* infection group and colonization group in the lung**

Indicators	Day 1	Day 2	Day 3	Day 5	Day 8	Day 10
WBC, $\times 10^9$/L						
Infection group	13.0 (8.1–19.1)	13.5 (7.4–20.1)	11.4 (7.5–18.4)	11.4 (6.4–17.4)	10.0 (8.0–16.9)	10.8 (7.3–18.5)
Colonization group	8.1 (5.4–12.3)	7.8 (6.7–11.4)	8.4 (6.4–12.2)	9.8 (6.7–11.4)	8.7 (7.0–10.3)	8.6 (6.9–11.0)
N(%)						
Infection group	89.0 (82.7–93.1)	88.9 (80.5–93.2)	89.3 (80.2–93.3)	85.5 (79.4–93.4)	86.0 (81.8–95.2)	85.4 (76.8–93.3)
Colonizing group	83.0 (73.9–86.3)	82.7 (73.6–87.9)	82.2 (74.6–87.8)	85.5 (79.4–93.4)	79.9 (70.3–88.0)	81.6 (69.4–87.8)
CRP, mg/L						
Infection group	76.0 (24.5–133.6)	77.8 (46.0–154.1)	62.5 (36.9–170.3)	82.0 (28.4–150.9)	76.0 (27.1–126.6)	60.0 (22.1–119.0)
Colonizing group	51.2 (14.2–91.4)	52.0 (13.1–96.5)	47.4 (14.2–81.6)	42.3 (16.8–79.1)	37.9 (15.5–88.8)	49.1 (16.5–111.4)
PCT, ng/mL						
Infection group	0.94 (0.20–2.51)	0.87 (0.32–3.23)	1.09 (0.39–2.76)	0.99 (0.42–2.13)	0.67 (0.34–1.19)	0.47 (0.27–0.90)
Colonizing group	0.14 (0.08–0.50)	0.15 (0.09–0.42)	0.13 (0.07–0.25)	0.11 (0.07–0.23)	0.09 (0.02–0.30)	0.47 (0.27–0.90)
sTREM-1, pg/mL						
Infection group	1,158.6 (779.5–1,582.3)	1,109.5 (722.2–1,726.4)	1,011.1 (802.5–1,817.5)	1,052.2 (691.0–1,729.8)	883.5 (685.7–1,268.2)	443.4 (291.7–602.3)
Colonizing group	532.8 (411.7–747.1)	480.8 (342.2–619.6)	450.4 (361.5–579.5)	500.8 (358.2–608.5)	443.4 (291.7–602.3)	469.2 (320.1–650.3)
T, °C						
Infection group	37.6 (37.2–38.0)	37.4 (37.1–38.0)	37.1 (37.0–37.8)	37.4 (37.1–37.7)	37.0 (36.8–37.5)	37.2 (36.8–37.6)
Colonizing group	37.5 (37.1–38.3)	37.6 (37.2–38.1)	37.8 (37.2–38.3)	37.2 (36.8–38.3)	37. (36.7–37.2)	36.9 (36.8–37.0)

Nonnormally distributed data are represented as "median (1/4 quartile–3/4 quartile)." There was a significant difference in WBC count between the infection group and the colonization group on the first day ($P < 0.05$), but no difference was found on the other days ($P > 0.05$).

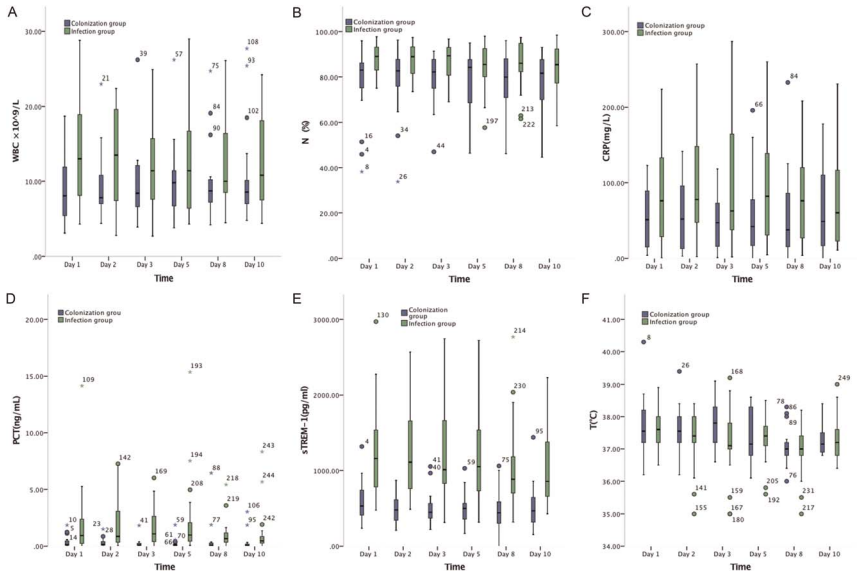

FIG. 1. **Dynamic monitoring of biomarkers in infection group and colonization group.** (A) Dynamic monitoring of WBC count in infection group and colonization group. (B) Dynamic monitoring of neutrophil ratio (N%) in infection group and colonization group. (C) Dynamic monitoring of CRP in infection group and colonization group. (D) Dynamic monitoring of PCT in infection group and colonization group. (E) Dynamic monitoring of sTREM-1 in infection group and colonization group. (F) Dynamic monitoring of body *T* in infection group and colonization group. CRP, C-reactive protein; PCT, procalcitonin; sTREM-1, soluble triggering receptor expressed on myeloid cells-1; T, temperature; WBC, white blood cell.

statistically different between infection and colonization groups ($P < 0.01$).

Serum levels of different biomarkers in infection and colonization groups

Dynamic changes of each index in infection and colonization groups are shown in Table 2 and Figure 1. Statistical differences were found in WBC on day 1 ($z = -2.225$, $P < 0.05$); N% on day 1($z = -2.610$, $P = 0.009$), day 2 ($z = -2.142$, $P = 0.032$), and day 3 ($z = -2.388$, $P = 0.014$); CRP on day 5 and in both sTREM-1 and PCT on days 1 to 10 ($P < 0.05$). Body temperature was not different between the two groups on any day ($P > 0.05$).

Receiver operating characteristic curve analysis of single and combined biomarkers for identification of infection and colonization

Significantly different biomarkers were further assessed by construction of ROC curves for sTREM-1, PCT, and N% on days 1, 2, and 3. Single and combined ROCs are presented in Table 3 and Figures 2 to 6. The combination of these three biomarkers improved diagnostic specificity and sensitivity.

There were significant differences in the proportion of neutrophils N% between the infection group and the colonization group on days 1 to 3 ($P < 0.05$). There was a significant difference in CRP between the infection group and the colonization group on the fifth day ($P < 0.05$), but there was no difference on the other days ($P > 0.05$). There were significant differences in PCT and STREM-1 between infection group and colonization group on days 1 to 10 ($P < 0.05$). There was no significant difference in body temperature *T* between infection group and colonization group on days 1 to 10 ($P > 0.05$).

On the first day, there were no significant differences in WBC count, N%, PCT, and sTREM-1 ($P > 0.05$). On day 2 and day 3, the ROC curves of N% and sTREM-1 were significantly different ($P < 0.01$). There was no significant difference in the ROC curves of PCT and sTREM-1 from day 1 to day 10 ($P > 0.05$), and there was no significant difference in the ROC curves of PCT and sTREM-1 from day 1 to day 10 ($P > 0.05$). On the first day, the ROC curves of combined "WBC &N&PCT &sTREM-1" were statistically different from those of single index (WBC count, N%, and PCT) ($P < 0.05$), but not from those of single index sTREM-1 ($P > 0.05$). On the second and third day, the ROC curve of combined "N&PCT &sTREM-1" was significantly different from that of single index N% ($P < 0.05$). The ROC curves of "PCT &sTREM-1" combined curve and single index (PCT and sTREM-1) had no statistical difference from day 1 to day 10 ($P > 0.05$), and the ROC curves of "PCT &sTREM-1" combined curve had no statistical difference ($P > 0.05$).

hcp analysis in Ab isolates

The presence of the *hcp* gene was detected by PCR in 44 of 96 Ab strains (45.83%) from lung specimens 25/35 (71.43%) from the infection and 19/61 (31.15%) from the colonization groups. *hcp* presence was significantly different between the two groups ($P < 0.05$) but the amount of *hcp* gene, calculated using the $2^{-\triangle\triangle ct}$ method, was not different ($P > 0.05$; Table 4).

mRNA of *hcp* was measured in the 44 hcp^+ Ab strains and no difference in transcript levels between infection and colonization groups was found ($P > 0.05$; Table 4).

DISCUSSION

Acinetobacter baumannii is a common clinical pathogen, which accounted for the top three respiratory tract isolates in 2016–2019, according to the China Antimicrobial Surveillance Network. Multidrug-resistant strains of HAP have become more frequent in clinical settings (14,19), contributing to a 52% Ab mortality rate (4). Hence, early identification of Ab colonization and infection is vital to prevent disease progression, reduce antibiotic abuse, and halt bacterial drug resistance.

TABLE 3. **Comparison of indicators for differentiation of pulmonary *A. baumannii* infection and colonization**

Index	AUC^{ROC}	Cutoff	Sensitivity	Specificity	95% CI	PPV, %	NPV, %	LR(+)	LR(–)
WBC									
Day 1	0.701	16.1	40.0	94.4	0.546–0.856	91	53	7.14	0.64
N									
Day 1	0.736	87.1	60.0	83.0	0.583–0.889	83.0	60.0	3.60	0.48
Day 2	0.693	88.7	52.0	89.0	0.534–0.853	87.0	57.0	4.68	0.54
Day 3	0.716	91.5	48.0	100.0	0.563–0.868	100.0	58.0	—	52.0
PCT									
Day 1	0.732	0.59	60.0	83.0	0.601–0.892	83.0	60.0	3.60	0.48
Day 2	0.808	0.75	64.0	89.0	0.674–0.937	89.0	64.0	5.76	0.41
Day 3	0.864	0.40	76.0	94.0	0.757–0.981	95.0	74.0	13.68	0.25
Day 5	0.900	0.20	96.0	78.0	0.800–1.000	86.0	93.0	4.32	0.05
Day 8	0.794	0.31	84.0	78.0	0.641–0.948	84.0	78.0	3.78	0.21
Day 10	0.831	0.20	92.0	83.0	0.686–0.977	88.0	88.0	5.52	0.10
sTREM-1									
Day 1	0.867	774.5	80.0	83.0	0.760–0.974	87.0	75.0	4.80	0.24
Day 2	0.929	648.9	88.0	83.0	0.857–1.000	88.0	83.0	5.28	0.14
Day 3	0.902	662.3	92.0	89.0	0.805–0.999	92.0	89.0	8.28	0.09
Day 5	0.883	583.4	88.0	78.0	0.783–0.983	85.0	82.0	3.96	0.15
Day 8	0.858	592.2	88.0	78.0	0.741–0.974	85.0	82.0	3.96	0.15
Day 10	0.847	647.1	84.0	78.0	0.724–0.969	84.0	78.0	3.78	0.21
WBC &N&PCT &sTREM-1									
Day 1	0.931	—	84.0	94.0	0.856–1.000	95.0	81.0	15.12	0.17
N&PCT &sTREM-1									
Day 2	0.951	—	88.0	94.0	0.889–1.000	96.0	85.0	15.84	0.13
Day 3	0.938	—	92.0	94.0	0.859–1.000	96.0	89.0	16.56	0.08
PCT &sTREM-1									
Day 1	0.902	—	84.0	89.0	0.809–0.995	91.0	80.0	7.56	0.18
Day 2	0.951	—	88.0	94.0	0.890–1.000	96.0	85.0	15.84	0.13
Day 3	0.929	—	96.0	89.0	0.844–1.000	92.0	94.0	8.64	0.05
Day 5	0.911	—	84.0	94.0	0.818–1.000	95.0	81.0	15.12	0.17
Day 8	0.864	—	88.0	78.0	0.751–0.978	85.0	82.0	3.96	0.15
Day 10	0.860	—	80.0	83.0	0.743–0.977	87.0	75.0	4.80	0.24

LR(+), positive likelihood ratio; LR(–), negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

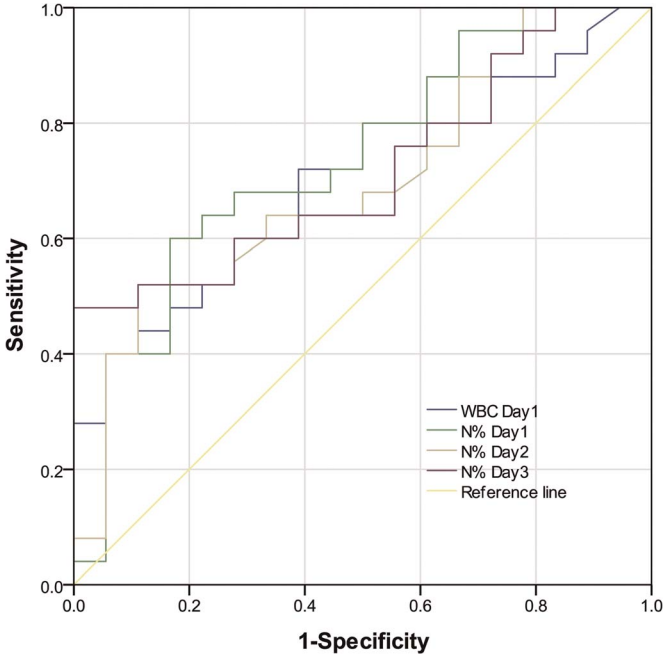

FIG. 2. **The ROC curves of WBC count on day 1 and N% on days 1–3 were used to differentiate infection group and colonization group.** ROC, receiver operating characteristic; WBC, white blood cell.

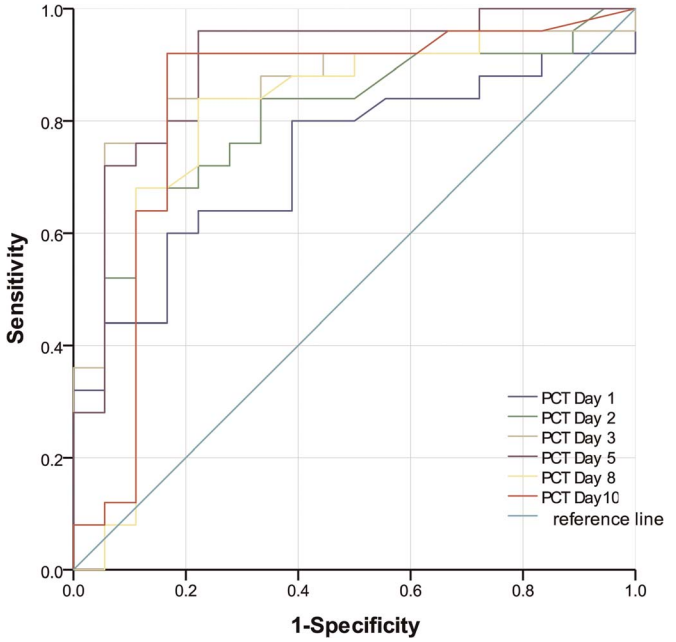

FIG. 3. **The ROC curves of PCT on days 1–10 were used to differentiate infection group and colonization group.** PCT, procalcitonin; ROC, receiver operating characteristic.

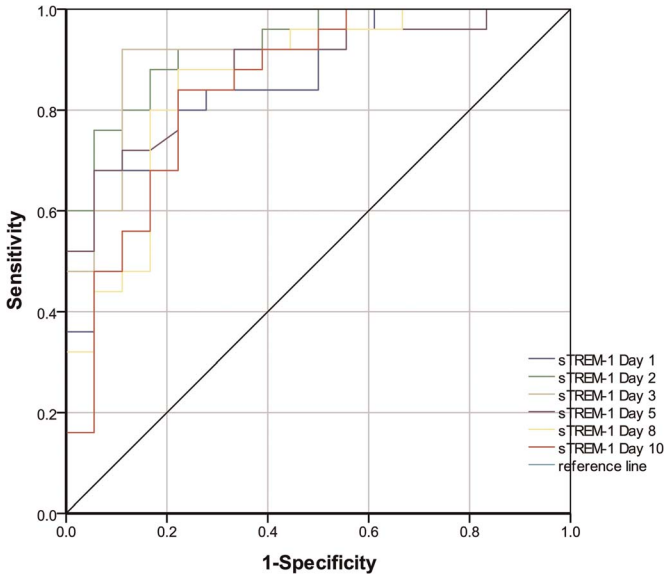

FIG. 4. **The ROC curves of sTREM-1 on days 1–10 were used to differentiate infection group and colonization group.** ROC, receiver operating characteristic; sTREM-1, soluble triggering receptor expressed on myeloid cells-1.

The current study found Ab infection rates to be 36.46% (35/96) and colonization rates to be 63.54% (61/96). Rates among the 53 cases (53/96, 55.21%) from the ICU, were 58.49% (31/53) infection and 41.51% (22/53) colonization. Variation among infection and colonization found at different hospitals has been reported (20). Martín-Aspas et al. (21) reported a 40% (49/122) Ab infection rate and a 60% (73/122) colonization rate while Rodríguez-Baño et al. (22) found Ab colonization to be approximately 50% in some Spanish hospitals. Differences may be attributed to local socioeconomic conditions, hospital management, epidemiological differences, and patients' basic conditions.

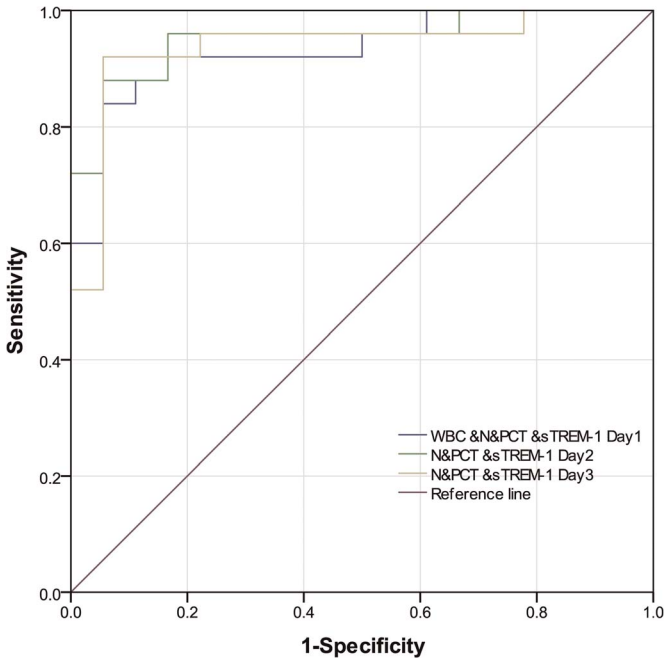

FIG. 5. **The ROC curves of multiple indicators on days 1–3 combined to identify infection and colonization.** ROC, receiver operating characteristic.

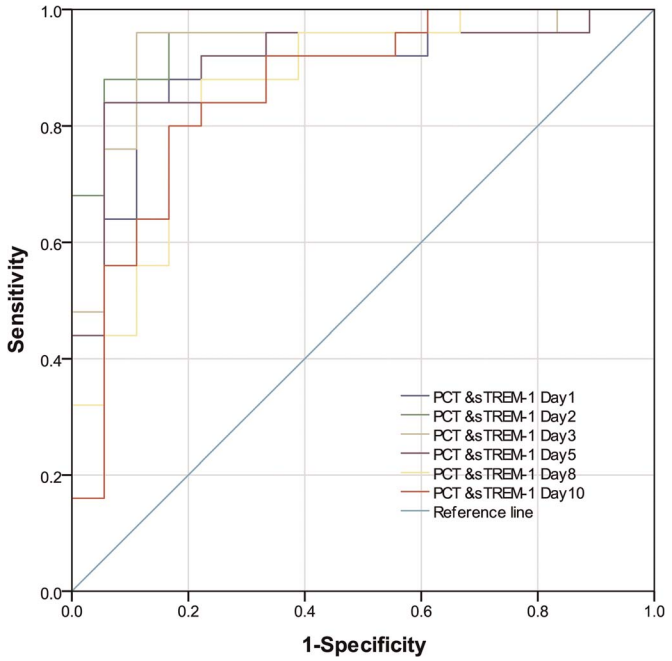

FIG. 6. **The ROC curves of the combination of sTREM-1 and PCT on days 1–10 for identifying infection and colonization.** PCT, procalcitonin; ROC, receiver operating characteristic; sTREM-1, soluble triggering receptor expressed on myeloid cells-1.

Higher Ab infection rates in ICUs than in other wards may be due to complications experienced by ICU patients, weakened immunity, more invasive treatments, and the closed nature of ICU wards, which renders them prone to aggregated infections. Moreover, the tracheotomy/intubation rate was significantly higher among the infection compared with the colonization group of the current cohort ($P < 0.05$), indicating that invasive operations of the respiratory tract constitute risk factors for Ab infection (16,23–25). Furthermore, the number of days of continuous Ab strain detection was greater for the infection group ($P < 0.01$). This observation indicates the possibility of distinguishing Ab infection and colonization based on duration of Ab detection. However, this method is time consuming, increasing patients' financial burdens and prolonging hospital stays. No significant differences were found between the two groups in terms of sex, age, CCI, antibiotic use within 3 months, or amounts of Ab. Thus, our preliminary conclusion is that a history of an ICU stay and invasive operations were the main risk factors for protracted lung Ab infection.

Microbial culture, WBC count and classification, CRP, PCT, and temperature monitoring are used to distinguish infection from colonization. A new inflammatory marker, sTREM-1 (8,16,26,27), for identifying bacterial infection and colonization in experimental animals has recently been reported (2). The current study found sTREM-1 and PCT to be significantly higher in the infection relative

TABLE 4. **Comparison of *hcp* gene quantity and *hcp* gene expression ($2^{-\Delta\Delta ct}$)**

Groups	Relative amount	*P*	Relative expression	*P*
Infection (n = 25)	0.53 (0.48–0.57)	> 0.05	0.51 (0.42–0.54)	> 0.05
Colonization(n = 19)	0.61 (0.53–0.77)		0.57 (0.47–0.76)	

Nonnormally distributed data are represented as "median (1/4 quartile–3/4 quartile)."

to the colonization group on days 1 to 10 ($P < 0.05$), demonstrating the potential utility of these two biomarkers. PCT and sTREM-1 ROC curves on days 1 to 10 were similar ($P > 0.05$), as were combined "PCT & sTREM-1" and single index (PCT or sTREM-1; $P > 0.05$). Thus, the combination of these two biomarkers possessed a similar test efficacy to individual indices in identifying Ab infection or colonization. Procalcitonin and sTREM-1 day 3 ROC curves had the highest positive likelihood ratios (13.68 and 8.28), indicating optimal diagnostic value. The sTREM-1 day 2 ROC curve showed the highest AUC value of 0.929 with optimal cutoff point 648.9 pg/L, sensitivity 88.0%, and specificity 83.0%. All data demonstrated the value of PCT and sTREM-1 for identifying lung Ab infection or colonization and early detection maximized their utility. Richard et al (28) also found an sTREM-1 AUC of 0.87 (95% CI, 0.81–0.92), superior to PCT and CRP and consistent with the current findings.

The N% on days 1 to 3 was significantly higher in the infection than in the colonization group ($P < 0.05$). N% AUC^{ROC}s on days 1, 2, and 3 were 0.736, 0.693, and 0.716, respectively, indicating that N% alone was not an ideal biomarker in distinguishing Ab infection from colonization. The WBC&N%&PCT &sTREM-1 AUC^{ROC} of 0.931 on day 1 was statistically different from individual index AUC^{ROC}s (WBC count, N%, and PCT; $P < 0.05$). N%&PCT&sTREM-1 AUC^{ROC} of 0.951 (day 2) and 0.938 (day 3) were significantly different from those for N% alone ($P < 0.05$) but not for sTREM-1 alone ($P > 0.05$). Thus, combined multiple indices improve the sensitivity and specificity of identifying lung Ab infection or colonization. This may be due to the excellent early-stage test efficacy of sTREM-1 and PCT, which is improved by addition of WBC count and N%. Moreover, variations in onset time, degree of increase, and duration affect inflammatory biomarkers individually, resulting in varied efficacy of markers in combination or individually.

White blood cell counts on day 1($z = -2.228$, $P = 0.026$) and CRP ($z = -1.994$, $P = 0.046$) on day 4 showed differences between the two groups, whereas no differences were found for temperature. These three indices have little utility for distinguishing Ab colonization from infection. Ito and Ishida (29) and Li et al. (30) have reported low specificity and sensitivity for WBC and both WBC and CRP can also increase during viral infection. Temperature fluctuates greatly in inpatients because of condition changes, coinfections, and anti-inflammatory treatment, giving it poor specificity. Elderly patients or patients with severe infections occasionally have normal temperature and WBC (31). Such studies illustrate the poor specificity of these three biomarkers, in agreement with our findings.

The T6SS is a gram-negative bacteria virulence system, which enables bacterial competition in the host, enhances invasiveness and adhesion, and improves bacterial viability within macrophages. T6SS is central to the pathogenesis of Ab infection (18–20,22,32,33). The T6SS marker, Hcp, shows different presence and expression levels in clinical isolates (11,14,15). We found a higher presence rate of the *hcp* gene in the infection (71.43%) than in the colonization group (31.15%) ($P < 0.05$), suggesting the utility of *hcp* presence in distinguishing Ab infection and colonization. Different studies have reported diverse *hcp* presence rates (11,14,15,33), perhaps related to local epidemiology, different sites of isolation, grouping criteria, and sample sizes. Presence and expression levels of *hcp* were similar in the two groups of the current cohort, which may reflect grouping criteria, multiple infections, and the sample size.

In summary, we propose that early-stage PCT and sTREM-1 values (within 3 days of Ab detection) may be used to identify Ab infection or colonization and combination with WBC and N % improves sensitivity and specificity. Dynamic monitoring of PCT and sTREM-1 aids the clarification of infection versus colonization. Detection of the *hcp* gene presence rate assists this process.

We acknowledge some limitations to the current study. First, no gold standard for distinguishing between pulmonary infection and colonization of *A. baumannii* exists. We can only use a widely used scoring tool reported in the literature, scored by two experienced attending physicians, which has potential bias. Second, the sample size was small and the subjects enrolled were from the same hospital, so that results may not be universally applicable. Third, ICU patients had complicated conditions influencing all infection biomarkers, which may affect the identification of Ab colonization and infection. Fourth, histological analysis is normally considered the criterion standard for diagnosis of pulmonary infections and the emphasis on clinical symptoms, imaging, and hematological examinations in the current study may have affected results. Fifth, *hcp* gene expression is influenced by many factors and their impact on identification of Ab infection and colonization has not been discussed. Lastly, lung Ab infection and colonization states are dynamic processes, as affected by bacterial virulence, drug resistance, human immunity, and medical level. Further investigations of the factors and mechanisms affecting host infection/colonization were required.

Therefore, it is of great significance that early identification of *A. baumannii* infection or colonization. As biomarkers (WBC, CRP, PCT, and sTREM-1) and body temperature are nonspecific indicators, the identification scheme proposed in this study from the aspect of body immunity has some false positives and false negatives. Therefore, this study explored the identification of lung Ab infection or colonization from the bacterial aspect (*hcp* gene carrier rate and its expression) and the body aspect (biomarkers and body temperature) and found that: (1) the identification of Ab infection and colonization requires consideration of high risk factors of infection (ICU hospitalization history and respiratory tract invasive operation) and the duration of infection/colonization; (2) the dynamic monitoring of blood biomarkers and body temperature has significance in distinguishing Ab infection and colonization, but there may still be the possibility of false positive and false negative; and (3) the carrier rate of *hcp* gene was higher in the infected group. In summary, biomarkers and the *hcp* gene are valuable for the identification of Ab infection or colonization in lung. Further exploration is required to verify the current findings.

ACKNOWLEDGMENTS

Authors' contributions: D.Y. did the conceptualization; X.W., J.J., C.W., W.Y., J.C., X.D. and D.Y. did the methodology; X.W., J.J., C.W., W.Y., J.C., X.D. and H.W. did the formal analysis; X.W., J.J., C.W., W.Y., J.C., X.D., H.W. and D.Y. did the investigation; X.W., J.J., and D.Y. did the writing—original draft; H.W., and D.Y. did the writing—review and editing; D.Y. did the project administration; D.Y., H.W. and X.W did the funding acquisition.

Availability of supporting data: The data in this article have not been published elsewhere and are available from the corresponding author upon request

REFERENCES

1. Weiner LM, Webb AK, Limbago B, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011–2014. *Infect Control Hosp Epidemiol*. 2016;37(11):1288–1301.
2. Hu C, Jiang J, Li Z, et al. Expression pattern of soluble triggering receptor expressed on myeloid cells-1 in mice with *Acinetobacter baumannii* colonization and infection in the lung. *J Thorac Dis*. 2018;10(3):1614–1621.
3. Mateo-Estrada V, Fernández-Vázquez JL, Moreno-Manjón J, et al. Accessory genomic epidemiology of cocirculating *Acinetobacter baumannii* clones. *mSystems*. 2021;6(4):e0062621.
4. Yu J, Tycksen E, Yang W, et al. Use of host response to refine the diagnosis of group A streptococcal pharyngitis. *J Pediatric Infect Dis Soc*. 2022;11(11):482–491.
5. Carrasco K, Boufenzer A, Jolly L, et al. TREM-1 multimerization is essential for its activation on monocytes and neutrophils. *Cell Mol Immunol*. 2019;16(5):460–472.
6. Jiang J, Wang X, Cheng T, et al. Dynamic monitoring of sTREM-1 and other biomarkers in acute cholangitis. *Mediators Inflamm*. 2020;2020:8203813.
7. Yu Y, Zhu C, Liu C, et al. Diagnostic performance of soluble triggering receptor expressed on myeloid cells-1 in ventilator-associated pneumonia of patients with ischemic stroke. *Can J Infect Dis Med Microbiol*. 2017;2017:9513690.
8. Feng JY, Su WJ, Pan SW, et al. Role of TREM-1 in pulmonary tuberculosis patients —analysis of serum soluble TREM-1 levels. *Sci Rep*. 2018;8(1):8223.
9. Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. *Front Immunol*. 2018;9:754.
10. Gao Q, Li Z, Mo X, et al. Combined procalcitonin and hemogram parameters contribute to early differential diagnosis of gram-negative/gram-positive bloodstream infections. *J Clin Lab Anal*. 2021;35(9):e23927.
11. Kim J, Lee JY, Lee H, et al. Microbiological features and clinical impact of the type VI secretion system (T6SS) in *Acinetobacter baumannii* isolates causing bacteremia. *Virulence*. 2017;8(7):1378–1389.
12. Fitzsimons TC, Lewis JM, Wright A, et al. Identification of novel *Acinetobacter baumannii* type VI secretion system antibacterial effector and immunity pairs. *Infect Immun*. 2018;86(8):e00297–e00218.
13. Ruiz FM, Santillana E, Spínola-Amilibia M, et al. Crystal structure of Hcp from *Acinetobacter baumannii*: a component of the type VI secretion system. *PLoS One*. 2015;10(6):e0129691.
14. Repizo GD. Prevalence of *Acinetobacter baumannii* strains expressing the type 6 secretion system in patients with bacteremia. *Virulence*. 2017;8(7):1099–1101.
15. Weber BS, Miyata ST, Iwashkiw JA, et al. Genomic and functional analysis of the type VI secretion system in *Acinetobacter*. *PloS One*. 2013;8(1):e55142.
16. Fan CY, Lee WT, Hsu TC, et al. Effect of chlorhexidine bathing on colonization or infection with *Acinetobacter baumannii*: a systematic review and meta-analysis. *J Hosp Infect*. 2019;103(3):284–292.
17. Ebrahimi ZS, Nasli-Esfahani E, Nadjarzade A, et al. Effect of symbiotic supplementation on glycemic control, lipid profiles and microalbuminuria in patients with non-obese type 2 diabetes: a randomized, double-blind, clinical trial. *J Diabetes Metab Disord*. 2017;16:23.
18. Wang F, An W, Zhang X. Copeptin combined with National Early Warning Score for predicting survival in elderly critical ill patients at emergency department. *Am J Emerg Med*. 2021;49:153–157.
19. Gao J, Zou Y, Wang Y, et al. Breath analysis for noninvasively differentiating *Acinetobacter baumannii* ventilator-associated pneumonia from its respiratory tract colonization of ventilated patients. *J Breath Res*. 2016;10(2):027102.
20. Di Venanzio G, Flores-Mireles AL, Calix JJ, et al. Urinary tract colonization is enhanced by a plasmid that regulates uropathogenic *Acinetobacter baumannii* chromosomal genes. *Nat Commun*. 2019;10(1):2763.
21. Martín-Aspas A, Guerrero-Sánchez FM, García-Colchero F, et al. Differential characteristics of *Acinetobacter baumannii* colonization and infection: risk factors, clinical picture, and mortality. *Infect Drug Resist*. 2018;11:861–872.
22. Pan P, Wang X, Chen Y, et al. Effect of Hcp iron ion regulation on the interaction between *Acinetobacter baumannii* with human pulmonary alveolar epithelial cells and biofilm formation. *Front Cell Infect Microbiol*. 2022;12:761604.
23. Rodríguez-Baño J, Cisneros JM, Fernández-Cuenca F, et al, Grupo de Estudio de Infección Hospitalaria (GEIH). Clinical features and epidemiology of *Acinetobacter baumannii* colonization and infection in Spanish hospitals. *Infect Control Hosp Epidemiol*. 2004;25(10):819–824.
24. Arvaniti K, Lathyris D, Ruimy R, et al. The importance of colonization pressure in multiresistant *Acinetobacter baumannii* acquisition in a Greek intensive care unit. *Crit Care*. 2012;16(3):R102.
25. Henig O, Weber G, Hoshen MB, et al. Risk factors for and impact of carbapenem-resistant *Acinetobacter baumannii* colonization and infection: matched case-control study. *Eur J Clin Microbiol Infect Dis*. 2015;34(10):2063–2068.
26. Gallop D, Scanlon KM, Ardanuy J, et al. Triggering receptor expressed on myeloid cells-1 (TREM-1) contributes to *Bordetella pertussis* inflammatory pathology. *Infect Immun*. 2021;89(10):e0012621.
27. Wang Y, Zhang S, Li L, et al. The usefulness of serum procalcitonin, C-reactive protein, soluble triggering receptor expressed on myeloid cells 1 and Clinical Pulmonary Infection Score for evaluation of severity and prognosis of community-acquired pneumonia in elderly patients. *Arch Gerontol Geriatr*. 2019;80:53–57.
28. Richard-Greenblatt M, Boillat-Blanco N, Zhong K, et al. Prognostic accuracy of soluble triggering receptor expressed on myeloid cells (sTREM-1)-based algorithms in febrile adults presenting to Tanzanian outpatient clinics. *Clin Infect Dis*. 2020;70(7):1304–1312.
29. Ito A, Ishida T. Diagnostic markers for community-acquired pneumonia. *Ann Transl Med*. 2020;8(9):609.
30. Li Y, Min L, Zhang X. Usefulness of procalcitonin (PCT), C-reactive protein (CRP), and white blood cell (WBC) levels in the differential diagnosis of acute bacterial, viral, and mycoplasmal respiratory tract infections in children. *BMC Pulm Med*. 2021;21(1):386.
31. Kushimoto S, Gando S, Saitoh D, et al, JAAM Sepsis Registry Study Group. The impact of body temperature abnormalities on the disease severity and outcome in patients with severe sepsis: an analysis from a multicenter, prospective survey of severe sepsis. *Crit Care*. 2013;17(6):R271.
32. Lopez J, Ly PM, Feldman MF. The tip of the VgrG spike is essential to functional type VI secretion system assembly in *Acinetobacter baumannii*. *mBio*. 2020;11(1):e02761–e02719.
33. Corral J, Pérez-Varela M, Sánchez-Osuna M, et al. Importance of twitching and surface-associated motility in the virulence of *Acinetobacter baumannii*. *Virulence*. 2021;12(1):2201–2213.