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# Dynamic cytokine profiles of bloodstream infection caused by *Klebsiella pneumoniae* in China

Wei Yu[1†], Linyan Zeng[2†], Xiang Lian[3], Lushun Jiang[1], Hao Xu[1], Wenhui Guo[1], Beiwen Zheng[1*] and Yonghong Xiao[1*]

## Abstract

**Objectives** The aim of this work was to assess dynamic cytokine profiles associated with bloodstream infection (BSI) caused by *Klebsiella pneumoniae* (Kpn) and investigate the clinical features associated with mortality.

**Methods** A total of 114 patients with positive BSI-Kpn and 12 sepsis individuals without blood positive bacteria culture were followed up. Cytokine profiles were analyzed by multiplex immunoassay on the first, third, seventh and fourteenth day after diagnosis. The test cytokines included arginase, interferon-gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-4, IL-6, IL-10, IL-12 (p70), and IL-23. The minimum inhibitory concentration (MIC) of 24 antibiotics were tested for BSI-Kpn. Risk factors associated with the 30-day mortality and 120-day mortality were evaluated using logistic analyses and nomogram.

**Results** There were 55 out of 114 patients with BSI-Kpn were included. All isolates showed high susceptibility rate to novel avibactam combinations. The level of arginase was the highest in carbapenem-resistant Kpn (CRKP) patients. The AUCs of arginase, TNF-α and IL-4 reached 0.726, 0.495, and 0.549, respectively, whereas the AUC for the combination of these three cytokines was 0.805. Notably, 120-day mortality in patients with CRKP was higher than carbapenem-sensitive *K. pneumoniae* (CSKP). Furthermore, the long-term and high levels of IL-6 and IL-10 were associated with death.

**Conclusions** High expression of arginase is correlated with CRKP. In addition, BSI-CRKP could result in indolent clinic course but poor long-term prognosis. Continuous increase of IL-6 and IL-10 were associated with mortality.

**Keywords** *Klebsiella pneumoniae*, Arginase, IL-6, IL-10, Mortality

[†]Wei Yu and Linyan Zeng contributed equally to this work.

*Correspondence:
Beiwen Zheng
zhengbw@zju.edu.cn
Yonghong Xiao
xiaoyonghong@zju.edu.cn
[1]State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, National Clinical Research Center for Infectious Diseases, Zhejiang University School of Medicine, Hangzhou, China
[2]Intensive Care Unit, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China
[3]Department of Infectious Diseases, Xiangshan First People's Hospital Medical and Health Group, The Affiliated Xiangshan Hospital of Wenzhou Medical University, Ningbo Fourth Hospital, Ningbo, China

## Introduction

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) infections has emerged as a global threat to public health. Bloodstream infection (BSI) caused by CRKP with regional differences are potentially life-threatening and severe patient-based disease burden [1, 2]. In China, BSI-CRKP has continued to increase from 7.0% in 2014 to 19.6% in 2019 [3]. Existing researches show that CRKP was a significant risk of excess mortality [4–6]. In addition, recent studies found CRKP induces a disease-tolerant immune response that is nonetheless often fatal [7, 8].

The early innate immune response to CRKP infection involves phagocytosis and clearance with inflammation. Cytokines play key roles in both innate and adaptive immune responses [9]. Intrapulmonary infection caused by CRKP is characterized by a deregulated lung immune response that resulted in excessive inflammation including cytokine storm, macrophage polarization and neutrophils accumulation [7, 10]. Several cytokines have been reported to mediate immune response against *K. pneumoniae* (Kpn), such as interferon-gamma (IFN-γ), interleukin (IL)-10 and IL-23 [8, 11]. However, a majority of evidence on the interplay between Kpn and the immune system was obtained by infecting rodents in vivo. Additionally, the effect of dynamic cytokine profiles on the inflammatory response induced by BSI-CRKP is less clear. Therefore, a retrospective cohort study was conducted to characterize the cytokine responses from patients with BSI-Kpn. Furthermore, factors associated with patient outcome were evaluated to provide a more accurate depiction of mortality predictors.

## Methods

### Study design and patients

We conducted a retrospective cohort study to obtain profiles of the immune response in patients with BSI-Kpn and identify the risk factors associated with short-term and long-term mortality BSI-Kpn. Clinical diagnosis of BSI-Kpn was screened by positive blood culture at The First Affiliated Hospital, Zhejiang University School of Medicine from May 2020 to June 2021. Human serum samples were collected on the first, third, seventh and fourteenth day after the onset of diagnosis. Serum samples of patients with sepsis were collected as control group. This study was approved by the recommendations of the Ethics Committee of The First Affiliated Hospital, Zhejiang University School of Medicine.

**Table 1** Minimum inhibitory concentrations of 24 antibiotics against BSI-Kpn

| Antibiotics | CRKP | | | ESBL-Kpn | | | S-Kpn | | |
|---|---|---|---|---|---|---|---|---|---|
| | MIC range (mg/L) | $MIC_{90}$ (mg/L) | *R* (%) | MIC range (mg/L) | $MIC_{90}$ (mg/L) | *R* (%) | MIC range (mg/L) | $MIC_{90}$ (mg/L) | *R* (%) |
| Cefazolin | > 128 | > 128 | 100.0% | > 128 | > 128 | 100% | 0.5->128 | 128 | 15.0% |
| Cefuroxime | 32->128 | > 128 | 100.0% | 32->128 | > 128 | 100% | 2->128 | 16 | 10.0% |
| Ceftriaxone | 16->128 | > 128 | 100.0% | 16->128 | > 128 | 100% | 0.03->128 | 0.25 | 5.0% |
| Ceftazidime | 2->128 | > 128 | 95.7% | 2-128 | 64 | 75% | 0.12-64 | 2 | 10.0% |
| Cefepime | 8->128 | > 128 | 95.7% | 2-128 | 128 | 83.3% | 0.016-16 | 0.125 | 5.0% |
| Cefoxitin | 32->128 | > 128 | 100.0% | 4-128 | 64 | 25.0% | 2-128 | 8 | 5.0% |
| Moxalactam | 8->128 | > 128 | 91.3% | 0.25-8 | 4 | 0.0% | 0,125-1 | 0.5 | 0.0% |
| Aztreonam | 8->128 | > 128 | 95.7% | 4->128 | 128 | 75% | < 0.016-64 | 1 | 5.0% |
| Ertapenem | 4->128 | > 128 | 100.0% | 0.016-1 | 0.5 | 0.0% | 0.016–0.25 | 0.125 | 0.0% |
| Meropenem | 2->32 | > 32 | 95.7% | 0.016–0.125 | 0.125 | 0.0% | 0.016–0.125 | 0.125 | 0.0% |
| Imipenem | 4->128 | > 128 | 95.7% | 0.125–0.25 | 0.25 | 0.0% | 0.06-4 | 2 | 5.0% |
| AMC | 16/8->128/64 | > 128/64 | 87.0% | 8/4–64/32 | 32/16 | 50.0% | 1/0.5–64/32 | 32/16 | 15.0% |
| TZP | 8/4->128/4 | > 128/4 | 82.6% | 2/4->128/4 | > 128/4 | 33.3% | 0.5/4->128/4 | 32/4 | 5.0% |
| CSL | 8/4->128/64 | > 128/64 | 87.0% | 4/2->128/64 | > 128/64 | 75% | 0.25/0.125-64/32 | 8/4 | 10.0% |
| CZA | 0.5/4->32/4 | 4/4 | 4.3% | 0.125/4-0.5/4 | 0.5/4 | 0.0% | 0.06/4-0.5/4 | 0.25/4 | 0.0% |
| AZA | 0.25/4 – 2/4 | 1/4 | - | 0.06/4-0.25/4 | 0.25/4 | - | 0.0125/4-0.125/4 | 0.125/4 | - |
| Gentamicin | 1->128 | > 128 | 87.0% | 1-128 | 2 | 16.7% | 0.5–128 | 2 | 10.0% |
| Amikacin | 2->128 | > 128 | 78.3% | 2–16 | 4 | 0.0% | 1–16 | 4 | 0.0% |
| Ciprofloxacin | 0.016->128 | > 128 | 91.3% | 1->128 | 64 | 100% | 0.016-128 | 32 | 30.0% |
| Levofloxacin | 0.125->128 | > 128 | 91.3% | 1->128 | 32 | 83.3% | 0.06-32 | 16 | 25.0% |
| Fosfomycin | 4->512 | 512 | 21.7% | 0.5–16 | 4 | 0.0% | 0.5–16 | 8 | 0.0% |
| Tigecycline | 0.125-32 | 0.5 | 4.3% | 0.125-2 | 0.25 | 0.0% | 0.125-1 | 0.125 | 0.0% |
| Polymyxin B | 0.5->32 | 2 | 8.7% | 0.5-1 | 1 | 0.0% | 0.5-1 | 1 | 0.0% |
| SXT | 0.5/9.5->8/152 | > 8/152 | 87.0% | > 8/152 | > 8/152 | 91.7% | 0.125/2.375->8/152 | > 8/152 | 20.0% |

MIC, minimum inhibitory concentration; S, susceptible; R, resistant; CRKP, Carbapenem-resistant *K. pneumoniae*; ESBL-Kpn, extended-spectrum β-lactamases producing *K. pneumoniae*; S-Kpn, susceptible *K. pneumoniae*; AMC, amoxicillin-clavulanic acid; TZP, piperacillin-tazobactam; CSL, cefoperazone-sulbactam; CZA, ceftazidime-avibactam; AZA, aztreonam-avibactam; SXT, Trimethoprim-sulfamethoxazol

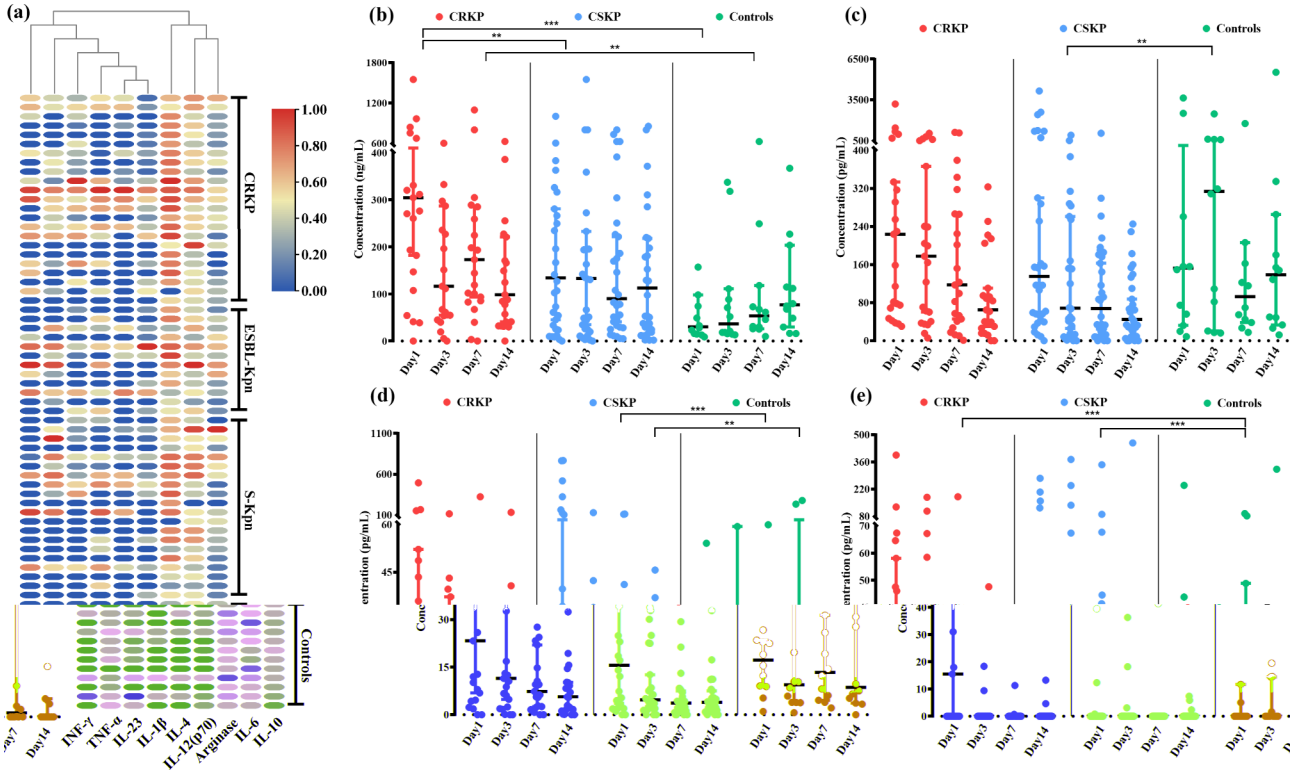


**Fig. 1** Serum cytokine levels in patients with BSI-Kpn and control group. (**a**) Heat map of serum cytokine concentrations for each patient. Colors represent high (red) or low (blue) concentration. (**b**). The concentration of arginase. (**c**) The concentration of IL-6. (**d**) The concentration of IL-10. (**e**) The concentration of IFN-γ. Error bars represent median with 95% CI. P scales of < 0.01 (***), < 0.05 (**). CRKP, Carbapenem-resistant *K. pneumoniae*; CSKP, carbapenem-sensitive *K. pneumoniae;* Controls, sepsis patients without blood positive culture

### Antibiotic susceptibility test

The minimal inhibitory concentration (MIC) of 24 antibiotics against BSI-Kp isolates were determined by agar dilution method, while polymyxin B was used broth dilution method. The details of antibiotics were consistent with our previous study [12]. The results for test antibiotics were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [13].

### Cytokine assay

Cytokine levels in serum were measured using the LEGENDplex™ Human Macrophage/Microglia Panel (catalog no. 740511; Biolegend, San Diego, CA, United States). Levels of 9 cytokines were measured, including arginase, IFN-γ, tumor necrosis factor alpha (TNF-α), IL-1β, IL-4, IL-6, IL-10, IL-12 (p70), and IL-23. All samples were assayed according to the manufacturer's instructions. Fluorescence intensity of the beads were acquired by CytoFLEX LX (Beckman Coulter Life Sciences, United States). Heat map analysis for cytokine data was performed using TBtools [14].

### Medical records

The medical records of included patients were reviewed. The data of laboratory examination on the first, seventh and fourteenth day were collected. The detailed analysis data and definitions were obtained as described in our previous study [4, 15]. The 30-day mortality and fellow-up for 120-day mortality were used as treatment outcomes.

### Statistical analysis

The results for abnormal distribution of continuous variables were presented as median (interquartile range) and analyzed using chi-square test. Multivariate analysis was performed after identifying variables with a *P*-value of < 0.05 in the univariate analysis. A two-tailed *P* value < 0.05 was considered to be statistically significant. GraphPad Prism software (V8.0) and SPSS 23.0 for Windows (SPSS Inc., Chicago, IL, USA) were used to analyze data. The nomogram for predicting prognosis of mortality was established basing on the regression model by employing the R package.

## Results

### Characteristics of included patients

A total of 114 patients with positive BSI-Kpn were followed up, of which 55 patients were included due to collecting blood samples on the first, third, seventh and fourteenth day after the onset of diagnosis. In addition,

12 sepsis patients without blood positive pathogens were included as controls. Among the positive BSI-Kpn results, there were 23 patients infected with CRKP, 12 patients infected with extended-spectrum β-lactamases (ESBL) producing *K. pneumoniae* (ESBL-Kpn) and 20 patients infected with non-CRKP or non-ESBL-Kpn considered as susceptible Kpn (S-Kpn). ESBL-Kpn and S-Kpn belong to carbapenem-sensitive *K. pneumoniae* (CSKP).

There were 47 patients (70.2%) were male. Mean age of patients with CRKP, CSKP and sepsis were 55±13, 56±18, 58±19 years, respectively. The most common department of patients with CRKP and sepsis is intensive care unit (ICU), while for CSKP is hepatobiliary surgery. Patients in CRKP group have been exposed to antibiotics before hospitalization. There is no statistical difference in Acute Physiology and Chronic Health Evaluation II score (APACHE-II score) and underlying disease among included patients (Supplementary Table 1).

#### Determination of MIC

A summary of 24 antibiotics MIC against BSI-Kpn is shown in Table 1. In total, 95.7%, 95.7% and 91.3% of CRKP isolates were susceptible to ceftazidime-avibactam, tigecycline and polymyxin B. All ESBL-Kpn isolates were susceptible to moxalactam, ceftazidime-avibactam, amikacin, fosfomycin, tigecycline and polymyxin B. The $MIC_{90}$ of aztreonam-avibactam for CRKP, ESBL-Kpn and S-Kpn were 1/4 mg/L, 0.25/4 mg/L and 0.125/4 mg/L, respectively.

#### Cytokine profiles among the patient groups

The arginase, IFN-γ, TNF-α, IL-1β, IL-4, IL-6, IL-10, IL-12 (p70), and IL-23 levels on the first, third, seventh and fourteenth day after the onset of diagnosis were assayed in all patients. The levels of test cytokines gradually decreased from the first day to fourteenth day among BSI-Kpn (Supplementary Fig. 1).

To compare differences in cytokine responses among different groups, overall cytokine data was clustered in a heat map representation (Fig. 1a; Table 2). A specific higher levels cluster of arginase, IL-6 and IL-10 in CRKP patients as compared to ESBL-Kpn, S-Kpn and sepsis patients. In particular, the level of arginase was the highest in CRKP patients compared with that in CSKP patients and controls ($P=0.002$) (Fig. 1b and Supplementary Fig. 1). The median cytokine levels of IL-6 on the third day were all higher in controls (313.7 pg/mL) as compared to CSKP (68.3 pg/mL) and CRKP (177.4 pg/mL) (Fig. 1c). IFN-γ and IL-10 levels were higher in the early acute phase (the first day) in CRKP as compared with CSKP and controls, although it did not reach statistical significance (Fig. 1d-e). However, in late phase (the seventh and fourteenth day), the levels of IFN-γ and IL-10 in patients with sepsis were higher than that in CSKP ($P<0.05$).

The correlation analysis between cytokines was performed to better understand the implication of cytokine profiles in the immune response in BSI-Kpn infection (Fig. 2a). Strong signature associated with IL-1β and IL-4 was present and significant correlations were observed between IL-1β and IFN-γ, as well as arginase and IL-23. The AUCs of arginase, TNF-α and IL-4 reached 0.726 (95% CI: 0.599–0.853), 0.495 (95% CI: 0.351–0.639), 0.549 (95% CI: 0.400-0.699), respectively, whereas the AUC for the combination of these three cytokines was 0.805 (95% CI: 0.702–0.909) (Fig. 2b).

#### Clinical features in patients with BSI-Kpn

Higher white blood cell count was observed in patients with CRKP on the first day after diagnosis ($P=0.05$) (Supplementary Tables 2 and Supplementary Fig. 2a). Hemoglobin and cholinesterase level in patients with CSKP was higher than CRKP and sepsis from the first day to the fourteenth day (Supplementary Fig. 2e-f). Markers of inflammation such as the percentage of neutrophils, high-sensitivity C-reactive protein (CRP) and procalcitonin (PCT) remained to be elevated in patients with sepsis ($P<0.01$) (Supplementary Fig. 2b-d). More patients in CRKP group have received continuous renal replacement therapy (CRRT) ($P=0.027$) and ceftazidime-avibactam treatment ($P<0.001$) (Supplementary Table 1).

#### Risk factors for 30-day mortality and 120-day mortality in enrolled patients

The level of arginase was the higher in survived patients compared with that in dead patients from the first day to the seventh day, although no significant difference was found (Table 3). It is of note that 120-day mortality in patients with CRKP was higher than that in CSKP and controls, while the 30-day mortality in CRKP were lower than CSKP (Fig. 3a-d). However, there was no significant difference in 30-day mortality and 120-day mortality between CRKP and CSKP groups ($P>0.05$). Among CRKP groups, 60.9% patients received definite treatment (tigecycline, polymyxin B, ceftazidime-avibactam) during 48 h after diagnosis, and 73.9% patients received ceftazidime-avibactam after diagnosis.

Lower hemoglobin and cholinesterase, higher CRP, total bilirubin and international normalized ratio on the fourteenth day showed significant associations with both 30-day mortality and 120-day mortality in a univariate analysis (Table 3 and Supplementary Table 3). Although no significant difference was found between the survivors and death regarding the arginase level, the arginase median level of 147.0 ng/mL on the first day in survivors was lower than that of 250.5 ng/mL in death during 30 days. Interestingly, persistent higher levels of IL-6 and

**Table 2** Cytokines profiles of individuals included in the study

| Indexes | Day1 | | | | Day3 | | | | Day7 | | | | Day14 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CRKP | CSKP | Controls | *P*-value | CRKP | CSKP | Controls | *P*-value | CRKP | CSKP | Controls | *P*-value | CRKP | CSKP | Controls | *P*-value |
| Arginase (ng/mL) | 304.7 (147.0-582.1) | 134.1 (27.5-323.8) | 30.3 (15.1–92.1) | 0.002 | 116.5 (53.7-297.2) | 133.2 (25.2-337.3) | 36.4 (16.2-105.3) | 0.208 | 173.0 (85.3-289.1) | 90.2 (30.4-244.7) | 53.6 (27.0-106.7) | 0.072 | 98.5 (40.6-227.4) | 112.5 (25.5-268.9) | 77.0 (34.5-181.3) | 0.713 |
| INF-γ (pg/mL) | 15.5 (0-64.6) | 0 (0-9.5) | 0 (0-10.1) | 0.219 | 0 (0-9.4) | 0 (0–0) | 0 (0-11.3) | 0.914 | 0 (0–0) | 0 (0–0) | 1.46 (0-39.5) | 0.009 | 0 (0–0) | 0 (0–0) | 0 (0-6.6) | 0.102 |
| TNF-α (pg/mL) | 3.6 (0-10.6) | 1.3 (0-35.5) | 4.4 (2.4–8.6) | 0.931 | 0.8 (0-8.4) | 0 (0-4.8) | 1.9 (0-7.3) | 0.26 | 0 (0–11.0) | 0 (0-13.1) | 4.0 (1.8–9.6) | 0.25 | 0.8 (0-10.2) | 0 (0–11.0) | 7.5 (0.6–17.1) | 0.173 |
| IL-1β (pg/mL) | 0 (0-20.9) | 0.7 (0-16.9) | 0 (0-2.9) | 0.267 | 0 (0-14.7) | 0 (0-6.5) | 0 (0-0.9) | 0.36 | 0 (0–10.0) | 0 (0-10.6) | 0 (0-5.5) | 0.866 | 0 (0-8.3) | 0 (0–7.0) | 1.1 (0-13.7) | 0.4 |
| IL-4 (pg/mL) | 0 (0-9.5) | 0 (0-6.7) | 0 (0-3.7) | 0.749 | 0.8 (0-15.6) | 0 (0-4.7) | 0 (0-1.3) | 0.281 | 2.4 (0-10.9) | 0 (0-6.6) | 2.2 (0-6.4) | 0.304 | 0 (0-6.4) | 0 (0-4.5) | 3.2 (0-10.7) | 0.419 |
| IL-6 (pg/mL) | 223.6 (68.6-680.7) | 135.0 (46.1-1075.1) | 152.5 (38.1-376.2) | 0.733 | 177.4 (40.5–504.0) | 68.3 (13.8-286.6) | 313.7 (35.9-589.1) | 0.095 | 117.2 (40.9-266.4) | 67.7 (17.7-179.7) | 92.5 (40.0-195.2) | 0.185 | 64.7 (26.1-204.8) | 44.7 (20.0-127.5) | 138.4 (37.2-243.89) | 0.168 |
| IL-10 (pg/mL) | 23.3 (4.6–73.1) | 15.6 (2.6–95.1) | 17.2 (8.9–23.4) | 0.882 | 11.5 (2.4–39.6) | 4.7 (2.1–22.6) | 9.4 (3.9–49.4) | 0.248 | 7.3 (2.4–23.3) | 3.7 (1.4–8.3) | 13.4 (4.9–30.0) | 0.056 | 5.6 (1.5–14.6) | 3.9 (1.9–10.3) | 8.6 (4.0-62.2) | 0.13 |
| IL-12 (p70) (pg/mL) | 1.6 (0.1–4.8) | 0.6 (0-3.8) | 0.4 (0-2.2) | 0.398 | 2.0 (0-3.3) | 1.0 (0–3.0) | 0.1 (0-1.1) | 0.293 | 0.9 (0-3.3) | 0.7 (0–5.0) | 0.3 (0-2.6) | 0.919 | 0.5 (0-1.5) | 0.8 (0-2.5) | 0.8 (0-2.2) | 0.689 |
| IL-23 (pg/mL) | 4.2 (0-14.5) | 0 (0-5.6) | 1.6 (0.2–7.2) | 0.056 | 3.9 (0-12.4) | 0.1 (0-4.4) | 1.9 (0.1–4.7) | 0.193 | 3.4 (0-11.6) | 0.2 (0-3.1) | 2.8 (0–15.0) | 0.169 | 2.0 (0-22.1) | 0 (0-2.9) | 1.8 (0.1–5.7) | 0.031 |

CRKP, carbapenem-resistant *K. pneumoniae*; CSKP, carbapenem-sensitive *K. pneumoniae*; Controls, sepsis patients without blood positive culture

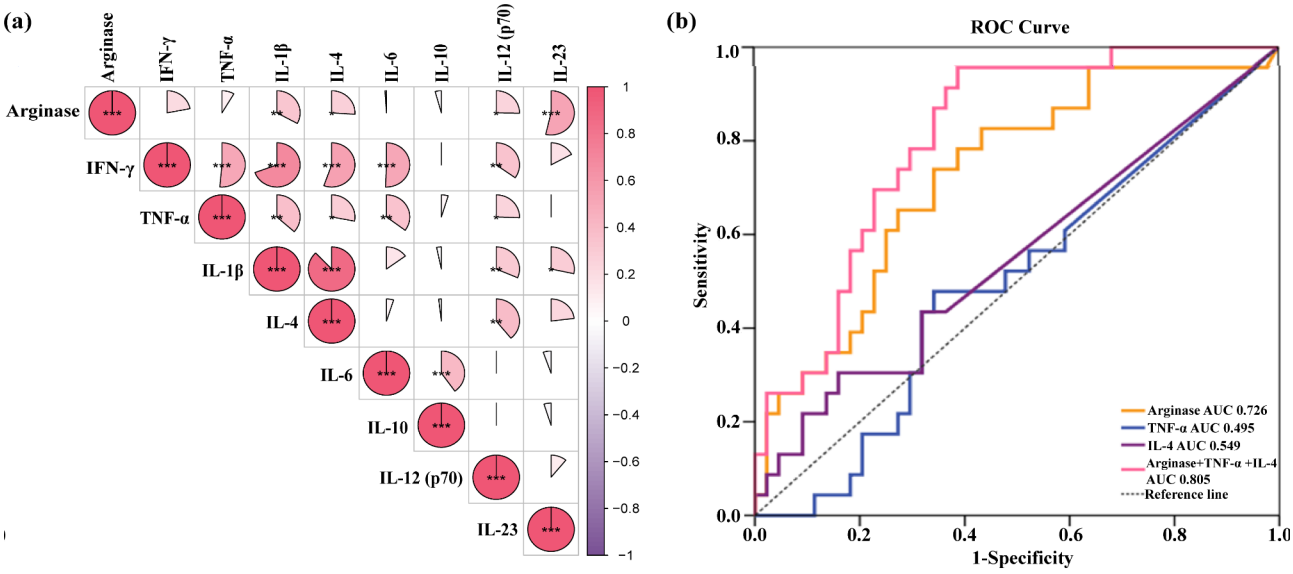


**Fig. 2** Correlations among concentrations of different cytokines and AUCs for CRKP. (**a**) Correlations among concentrations of different cytokines. (**b**) AUCs of arginase, TNF-α and IL-4 for CRKP

IL-10 were associated with higher 30-day mortality and 120-day mortality (Table 3 and Supplementary Table 3).

Variables were further analyzed using logistic regression for 30-day mortality. The multivariate predictive model was established, including cholinesterase on the first day, CRP and hemoglobin on the seventh day, total bilirubin, IL-6 and IL-10 on the fourteenth day, underlying disease (immunosuppressant usage, tumor, coronary disease), and polymyxin B treatment after diagnosis (Supplementary Table 4). The weight of IL-6 level is maximum. The sum of each factor scores could predict mortality risk (Fig. 3e).

## Discussion

BSI-Kpn, especially for CRKP, was associated with high morbidity and mortality [4–6]. Fortunately, several novel antibiotics have been developed to combat CRKP [16]. Recent studies have recognized that pro-inflammatory signalling is crucial to Kpn clearance. Therefore, boosting innate defence is an attractive approach to exploit new therapeutics against BSI-Kpn [17]. In the present study, we found a strong arginase signature is associated with BSI-CRKP. Our results showed early effective treatment such as ceftazidime-avibactam and CRRT may extended survival time of CRKP during early infection stage, however, the mortality increased with time. Persistent higher levels of IL-6 and IL-10 would cause higher mortality.

In this study, BSI-CRKP showed high susceptibility rate to novel avibactam combinations, which is consistent with previous results [18]. However, there were still 4.3% CRKP were resistant to ceftazidime-avibactam. Although the susceptibility breakpoint for aztreonam-avibactam has not been approved, the $MIC_{90}$ remain low level. Thus, novel antibiotics bring hope to get rid of limited treatment dilemma.

The published evidence establishes that different cytokines is critical for host defence against Kpn [7–9, 17]. Cytokines could be expressed by many cells of the immune system, and further control the activation of innate immune responses. However, few studies have addressed the relationship between antibiotic resistance and inflammatory response. We chose a panel of cytokines related to macrophage polarization, as well as regulatory states: Th1 immune response (IFN-γ), Th2 (IL-4), Th17 (IL-23), regulatory immune functions (IL-10) and adaptive immunity (IL-1β, IL-6, IL-12 (p70)) [19]. The results revealed the levels of arginase, IL-6 and IL-10 in patients with CRKP was higher during BSI early stage. In addition, arginase may be conducive to distinguish CRKP from CSKP. The summarised evidence suggest Kpn could exploit IL-10 to attenuate immune response [8]. Similarly, arginase expression play an essential role in immunomodulation [20, 21]. Current studies demonstrated arginase is an important marker of alternative anti-inflammatory polarization of macrophages to limit the exaggerated inflammatory response during infections [20, 22] Moreover, it has been also suggested arginase-2 was a downstream mediator of IL-10 and was essential for skewing mitochondrial dynamics in inflammatory in vivo [21]. Thus, CRKP may down-regulate the level of inflammation, resulting in long-term residence in host. The complex cytokines microenvironment, composition changes and further immune responses are radically different depending on various factors over time. However, the overall trend of test cytokines in this study were decreased from the first

**Table 3** Cytokines profiles and laboratory testing associated with 30-day mortality

| Indexes | Day1 | | | Day3 | | | Day7 | | | Day14 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Survivors | Death | *P*-value | Survivors | Death | *P*-value | Survivors | Death | *P*-value | Survivors | Death | *P*-value |
| Arginase (ng/mL) | 147.0 (41.0-330.9) | 250.5 (15.4–450.0) | 0.987 | 71.0 (24.9–287.0) | 152.8 (24.7-308.4) | 0.731 | 101.8 (39.8-250.8) | 97.1 (33.6-459.4) | 0.851 | 98.5 (32.2-220.1) | 73.6 (29.3-332.1) | 0.695 |
| INF-γ (pg/mL) | 0.0 (0-39.5) | 0 (0-103.5) | 0.528 | 0 (0-1.7) | 0 (0-7.1) | 0.806 | 0 (0–0) | 0 (0–0) | 0.622 | 0 (0–0) | 0 (0–0) | 0.281 |
| TNF-α (pg/mL) | 3.1 (0-12.1) | 5.9 (0.6–51.0) | 0.335 | 0 (0-8.3) | 2.4 (0-7.6) | 0.471 | 0 (0-9.6) | 3.7 (0-16.2) | 0.257 | 0 (0-12.8) | 2.9 (0-9.8) | 0.944 |
| IL-1β (pg/mL) | 0 (0-13.7) | 0.1 (0-22.4) | 0.832 | 0 (0-6.9) | 0 (0–0) | 0.261 | 0 (0-9.3) | 0 (0-23.9) | 0.941 | 0 (0-8.2) | 0 (0-11.6) | 0.794 |
| IL-4 (pg/mL) | 0 (0-3.9) | 0 (0-7.4) | 0.381 | 0 (0–4.0) | 3.3 (0-13.1) | 0.201 | 0 (0-7.8) | 0 (0-20.3) | 0.817 | 0 (0-5.6) | 1 (0-19.8) | 0.488 |
| IL-6 (pg/mL) | 116.7 (43.5-680.7) | 160.3 (79.5-2499.7) | 0.273 | 69.6 (20.6-287.8) | 409.7 (216.9-824.3) | 0.001 | 84.9 (24.6-185.7) | 103.6 (47.4-323.9) | 0.160 | 42.7 (19.1-109.9) | 193.8 (102.5-317.2) | 0.000 |
| IL-10 (pg/mL) | 12.8 (2.8–54.1) | 23.2 (11.6–66.3) | 0.150 | 5.1 (2.0-16.9) | 27.7 (9.6–66.5) | 0.003 | 4.3 (1.6–10.6) | 25.2 (12.0-71.7) | 0.001 | 3.9 (1.5–8.3) | 18.3 (9.8-116.8) | 0.000 |
| IL-12 (p70) (pg/mL) | 0.8 (0-3.8) | 0.5 (0–5.0) | 0.790 | 0.7 (0-2.7) | 0.8 (0-6.3) | 0.633 | 0.5 (0-2.5) | 2.1 (0.2–11.2) | 0.060 | 0.7 (0-2.3) | 0.2 (0-4.1) | 0.973 |
| IL-23 (pg/mL) | 1.9 (0-9.3) | 0.8 (0-3.9) | 0.579 | 0.9 (0-8.6) | 1.9 (0-3.7) | 0.879 | 1.0 (0-11.1) | 1.3 (0-7.2) | 0.846 | 1.3 (0-5.7) | 0.7 (0-2.7) | 0.684 |
| WBC (*10E9/L) | 8.5 (5.3–14) | 10.3 (1.5–16.5) | 0.967 | | | | 7.4 (4.8–10.8) | 10.4 (2.8–12.0) | 0.844 | 7.0 (4.8–10.0) | 7.5 (3.1–15.6) | 0.719 |
| N (%) | 88.3 (82.0-92.6) | 91.8 (86.1–96.2) | 0.139 | | | | 82.1 (72.9–86.7) | 84.6 (54.3–92.7) | 0.545 | 78.9 (68.0-85.1) | 88.4 (60.8–90.7) | 0.185 |
| Hb (g/L) | 94.0 (72.0-117.0) | 80.5 (65.3–89.3) | 0.023 | | | | 82.0 (70.0-104.0) | 65.5 (63.3–70.8) | 0.001 | 78.0 (70.0–99.0) | 66.0 (52.5–76.3) | 0.004 |
| PLT (*10E9/L) | 103.0 (53.0-170.0) | 55.0 (11.8-238.5) | 0.452 | | | | 117.0 (50.0-224.0) | 65.0 (38.8-205.5) | 0.208 | 211.0 (107.0-278.0) | 58.5 (11.5-199.5) | 0.004 |
| CRP (mg/L) | 68.0 (27.9-131.4) | 107.3 (42.8-171.7) | 0.423 | | | | 38.4 (12.9–68.8) | 90.5 (63.3–111.0) | 0.010 | 22.6 (6.4–47.3) | 81.5 (48.1-131.2) | 0.002 |
| PCT (ng/mL) | 1.7 (0.6–11.5) | 1.2 (0.6–9.6) | 0.744 | | | | 0.4 (0.2-1.0) | 0.8 (0.6–1.4) | 0.089 | 0.3 (0.2–0.6) | 0.9 (0.4–1.9) | 0.012 |
| Alb (g/L) | 32.9 (27.6–36.8) | 31.2 (29.1–36.5) | 0.624 | | | | 33.1 (31.0-37.2) | 34.2 (29.0-37.5) | 0.864 | 35.2 (32.7–38.1) | 34.1 (29.5–36.2) | 0.236 |
| ALT (U/L) | 29.0 (15.0–63.0) | 45.5 (26.0-110.5) | 0.082 | | | | 28.0 (18.0–44.0) | 32.0 (21.3–54.8) | 0.368 | 26.0 (17.0–41.0) | 20.5 (13.8–34.0) | 0.452 |
| AST (U/L) | 30.0 (16.0–60.0) | 47.0 (25.3–92.0) | 0.098 | | | | 24.0 (16.0–42.0) | 40.0 (28.0–63.0) | 0.042 | 23.0 (16.0–38.0) | 25.5 (18.3–71.0) | 0.310 |
| CHE (U/L) | 3154 (2373.0-4523.0) | 2251.5 (1285.0-2572.0) | 0.007 | | | | 3094.0 (2137.0-4522.0) | 2546.5 (1157.3-3025.8) | 0.024 | 3241.0 (2174.0-4346.0) | 1330.0 (1157.0-2544.8) | 0.001 |
| Tbil (μmol/L) | 20.1 (9.8–35.6) | 41.3 (16.5-186.4) | 0.048 | | | | 16.3 (11.2–30.7) | 62.2 (26.1–80.2) | 0.001 | 14.5 (9.4–30.3) | 69.8 (21.5–130.0) | 0.001 |
| Cr (μmol/L) | 77.0 (54.0–99.0) | 90.0 (46.8–154.0) | 0.967 | | | | 67.0 (47.0–93.0) | 63.5 (39.8-121.5) | 0.864 | 64.0 (48.0–92.0) | 60.5 (39.0-148.8) | 0.665 |
| INR | 1.1 (1.1–1.2) | 1.3 (1.1–1.4) | 0.047 | | | | 1.2 (1.1–1.3) | 1.3 (1.1–1.6) | 0.145 | 1.2 (1.1–1.3) | 1.4 (1.2–1.7) | 0.006 |

WBC, white blood cell; N, Percentage of neutrophils; Hb, hemoglobin; PLT, platelet; CRP, high-sensitivity C-reactive protein; PCT, procalcitonin; Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHE, cholinesterase; Tbil, total bilirubin; Cr, creatinine; INR, international normalized ratio

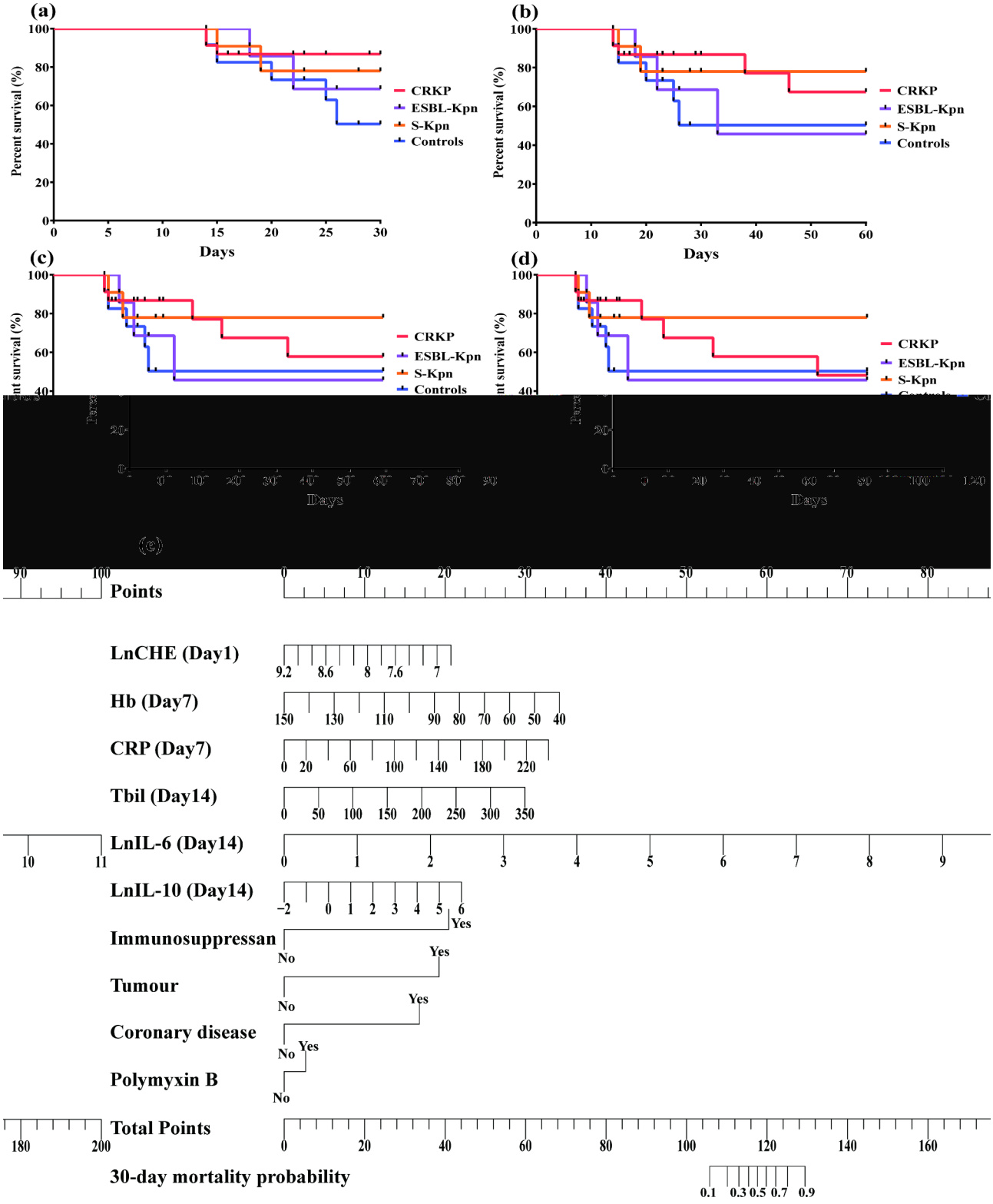


**Fig. 3** Survival rate of enrolled patients. (**a**) 30-day mortality. (**b**) 60-day mortality. (**c**) 90-day mortality. (**d**) 120-day mortality. (**e**) Nomogram for predicting the 30-day mortality. CRKP, Carbapenem-resistant *K. pneumoniae*; ESBL-Kpn, extended-spectrum β-lactamases producing *K. pneumoniae*; S-Kpn, susceptible *K. pneumoniae;* Controls, sepsis patients without blood positive culture

day to fourteenth day after diagnosis. Exploration of the dynamic immunomodulatory role of cytokines deserve

further attention.

It has been well-documented that CRKP was associated with mortality [4–6]. Our results showed 30-day mortality in patients with BSI-CRKP was lower than that in CSKP and controls, which is inconsistent with previous study [6]. This was probably because there was no significant difference in APACHE-II score between CRKP and CSKP groups in the present study. In addition, a majority of patients with CRKP received timely and effective antibiotics, especially for ceftazidime-avibactam. Previous study also demonstrated ceftazidime-avibactam was an independent favorable prognostic factor for 30-day mortality [4]. However, the mortality in CRKP gradually increased with time prolonging. This may be related to CRKP could elicit immune responses tolerance in early infection stage [7, 8]. Additionally, the effect of CRKP on mortality was probably influenced by inadequate and unnecessarily broad empiric antibiotics [23]. A total of 78% CRKP patients accepted ceftazidime-avibactam after diagnosis. Therefore, both immune responses and antibiotics played an important role in survival. Of note, IL-6 and IL-10 overexpression resulted in more pronounced bacteraemia and accelerated mortality in Kpn infected mice [24, 25]. Our results are consistent with these observations. To obtain comprehensive information, it will be necessary to assess the interaction among BSI-Kpn, antibiotics and host immune responses.

This study firstly provides an insight into dynamic cytokine profiles of BSI-Kpn. However, there were also several limitations in this study. First, the number of included patients was limited. Subpopulations of immune cells associated with BSI-Kpn did not do further analysis. Moreover, our study did not cover the direct relationship between immune cells and cytokines. However, the results clearly show significant changes of cytokines and clinical features during BSI-Kpn, providing valuable information to explore the hypothesis regarding the BSI-Kpn mediated immune evasion of macrophage in early infection stage.

## Conclusions

In conclusion, high expression of arginase could be as a promising biomarker for early diagnosis of CRKP. In addition, CRKP could induce more indolent course of BSI by mediating macrophage-derived cytokines. Furthermore, persistent higher levels of IL-6 and IL-10 were associated with mortality. Further randomized, double blind controlled trials are warranted to validate these findings.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12941-024-00739-7.

**Supplementary Material 1**: **Supplementary Fig. 1.** Serum cytokine levels in different BSI-Kpn groups. (a) arginase; (b) IFN-γ; (c) TNF-α; (d) IL-1β; (e) IL-4; (f) IL-6; (g) IL-10; (h) IL-12 (p70); (i) IL-23.

**Supplementary Material 2**: **Supplementary Fig. 2.** Clinical features in patients with BSI-Kpn. (a) White blood cell (WBC). (b) Percentage of neutrophils (N). (c) High-sensitivity C-reactive protein (CRP). (d) Procalcitonin (PCT). (e) Hemoglobin (Hb). (f) Cholinesterase (CHE). CRKP, Carbapenem-resistant *K. pneumoniae*; ESBL-Kpn, extended-spectrum β-lactamases producing *K. pneumoniae*; S-Kpn, susceptible *K. pneumoniae;* Controls, sepsis patients without blood positive culture.

**Supplementary Material 3**

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None.

**Author contributions**
The work presented here was carried out in collaboration between all authors. WY, BWZ and YHX developed the concept and designed the study. WY and LYZ collected the samples. LYZ, XL and LSJ completed antibiotic susceptibility test. Cytokine assay was measured WY, LSJ and HX. Medical records were analyzed by LZY, WHG and BWZ. The manuscript was written by WY and corrected by BWZ and YHX. All authors discussed the results and implications and commented on the manuscript at all stages.

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**Data availability**
No datasets were generated or analysed during the current study.

## Declarations

**Ethics approval and consent to participate**
This study was approved by the recommendations of the Ethics Committee of The First Affiliated Hospital, Zhejiang University School of Medicine with written informed consent from all subjects (Reference Number: 2021789).

**Competing interests**
The authors declare no competing interests.

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## Publisher's note