ORIGINAL ARTICLE

Development of a Simple Score for Diagnosis Melioidosis

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Background: Melioidosis is common gram negative bacterial infection in northeastern Thailand caused by *Burkholderia pseudomallei*. Patients often experience severe conditions and high mortality rates.

Objective: To develop a clinical prediction model to estimate the risk of melioidosis septicemia.

Materials and Methods: This retrospective case-control study included patients with positive hemoculture for *Burkholderia pseudomallei* (BP) and other gram-negative bacteria (*Escherichia coli*, and *Klebsiella pneumoniae*) admitted to Srinagarind Hospital between January 2015 and December 2020. Logistic regression analyses were used to determine the calculation of a score for diagnosing melioidosis infection.

Results: A total of 426 patients with positive hemoculture were included: 132 patients for BP and 294 patients for other gram-negative bacteria. The clinical prediction model for diagnosing melioidosis utilized seven variables: age ≥60 years (-2 points), male gender (3 points), duration of symptom onset to hospitalization ≥7 days (5 points), occupation as a farmer (3 points), presence of diabetes mellitus (2 points), presence of cancer (-5 points), and platelet count (x109/L) (200 to 399.9: 1 point, ≥400: 3 points). The model demonstrated good discrimination (area under the curve: 0.89; 95% CI: 0.86 to 0.93) and acceptable calibration (Hosmer and Lemeshow goodness of fit test: p=0.252). A cut-off point of the melioidosis score ≥5 points (maximum score=16 and minimum score = -7) resulted in an accuracy of 84.3% (95% CI 80.5 to 87.6), a sensitivity of 78.8%, and a specificity of 86.7%.

Conclusion: The melioidosis score exhibited high performance and clinical utility in predicting melioidosis infection.

Keywords: Melioidosis; *Burkholderia pseudomallei*; Diagnostic score; Clinical prediction model; Gram-negative bacteria

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Diagnostic prediction models are employed to estimate the presence or absence of a disease in an individual based on multiple predictor values. Frequently developed using regression techniques such as logistic and Cox regression, these models personalized healthcare recommendations and informed decision-making. A higher prediction score signifies an elevated risk of disease, while a lower score denotes a reduced risk.

Infections caused by gram-negative bacteria, such as *Escherichia coli*, and *Klebsiella pneumonia*, are widespread in both community-acquired and hospital-acquired cases. The mortality rate is approximately 49.7%(1). *Burkholderia pseudomallei*, another gram-negative bacterium endemic to the Northeast of Thailand, is the most common cause of community-aquired infection, resulting in melioidosis.

Burkholderia pseudomallei is a gram-negative, rod-shaped bacterium found on soil surfaces and natural water sources. Transmission can occur through skin contamination and inhalation(2). Melioidosis can affect various body parts, such as the lungs, liver, spleen, kidneys, skin, connective tissue, bones, and joints(3). Disseminated melioidosis or bloodstream infection results in a mortality rate of 54.2%(4). Infected individuals often present with nonspecific symptoms and lack specific laboratory tests, making it challenging to differentiate melioidosis from other gram-negative bacterial infections. Previous studies have investigated risk factors for *B. pseudomallei* infection.

An Australian study identified factors influencing melioidosis infection, including diabetes mellitus, alcohol use, and chronic kidney disease. A Thai study found that contributing factors encompassed diabetes, chronic kidney disease, thalassemia, and agricultural-related occupations

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with exposure to soil and water[5]. However, the only statistically significant factor was diabetes[6]. Melioidosis can develop even in patients without risk factors. Early diagnosis and appropriate antibiotic treatment are crucial for successful melioidosis management. Misdiagnosis is common, as symptoms resemble those of other infections.

The present study was to develop a melioidosis score that can accurately and swiftly identify patients with melioidosis. This tool could consider a combination of factors such as clinical symptoms, laboratory results, and exposure history, to assess the likelihood of melioidosis infection, thereby aiding healthcare providers in making prompt and accurate diagnoses.

Materials and Methods

Study setting and patient population

The present study was a 5-year retrospective case-control study conducted at Srinagarind Hospital, Khon Kaen University, which is a tertiary academic hospital in Thailand. The study period was from January 2015 to December 2020. All adult patients (≥18 years old) who were diagnosed with melioidosis from positive blood culture were included in the study. Furthermore, we also included all adult patients who were diagnosed with other gram-negative bacteremias (positive blood culture of *Escherichia coli* and *Klebsiella pneumoniae*) within 48 hours before or after hospital admission. The study was approved by the Center for Ethics in Human Research at Khon Kaen University (EC number: HE661072).

Patients with positive blood cultures for *Escherichia coli* and *Klebsiella pneumoniae* were 1,069 and 419, respectively. The authors performed a random sampling of 154 patients in each group of other gram-negative bacteremias.

Data collection and definition

Baseline characteristics, physical examination findings, and laboratory investigations were recoded for all eligible patients. Baseline characteristics included age, sex, body mass index (BMI), occupation, hometown, comorbidity, alcohol consumption, level of immunosuppression, the Acute Physiology and Chronic Health Evaluation (APACHE) III score, and Sequential Organ Failure Assessment (SOFA) score. Physical examination included vital signs and the Glasgow Coma Scale score. Laboratory investigations included a complete blood count, kidney function, liver function test, serum glucose, HbA1C, serum lactate, arterial blood gas, and melioidosis titer. Study data were collected and managed using REDCap (Research Electronic Data Capture), which is a secure, web-based software platform designed to support data capture for research studies and hosted at Khon Kaen University.

Statistical analysis

Potential predictors were stratified by melioidosis and other gram-negative bacteremia. Continuous variables were summarized using mean ± standard deviation (SD) or median and interquartile range (IQR), depending on the data normality. Categorical variables were described using counts and percentages (%). Two-sample t-test or Wilcoxon rank-sum test was used to compare continuous variables. The Chi-square test or Fisher's exact test was used for categorical variables. Univariable comparisons of each predictor were conducted using logistic regression. Variables with $p<0.1$ in univariate analyses that were associated with melioidosis were included in the multivariable model. Multicollinearity among candidate predictors was evaluated using correlation testing and variable inflation factor.

The authors developed a multivariable model predicting melioidosis using logistic regression. For internal validation, the authors used 20 repeats of 4-fold cross-validation. A score was constructed to predict melioidosis and enable individual prediction. Model performance was evaluated using the area under the curve (AUC). The cut-off point for melioidosis diagnosis was determined using Youden index.

To evaluate the calibration of the scoring, the authors assessed the calibration curve, and the goodness of fit was evaluated using the Hosmer-Lemeshow test. A $p>0.05$ indicates an acceptable model fit. Decision curve analysis (DCA) was conducted to assess the clinical usefulness of the developed clinical prediction model for identifying patients at risk for melioidosis. The authors conducted statistical analyses in R version 4.2.2, and significance was evaluated at the 0.05 level using a two-sided test.

Results

A total of 426 patients who met the study criteria were included in the analysis. Among these, 294 patients (68.9%) were diagnosed with a gram-negative bacterial infection caused by *Escherichia coli* and *Klebsiella pneumoniae*, while 132 patients (31.1%) were diagnosed with melioidosis caused by *B. pseudomallei*.

Model development

The model was developed to investigate the clinical and laboratory features to predict culture-positive melioidosis. The median age of the 132 culture-positive melioidosis patients was 58 years, with a majority of patients being male (104 patients; 78.8%). Among the melioidosis patients, farmers constituted the most common occupation (61 patients; 46.2%). The most frequently occurring comorbidity of melioidosis was diabetes mellitus (DM), observed in 75 patients (56.8%), while patients with a history of malignancies had more occurrence of other gram-negative septicemia. The severity of illness among melioidosis patients was less severe, and sepsis, as shown in Table 1.

The onset of symptoms to the hospitalization in melioidosis patients was longer than in other gram-negative septicemia groups, and skin and soft tissues infection were common in melioidosis. Conversely, patients with other gram-negative septicemia had more common primary bacteremia and urinary tract infections. For vital signs, melioidosis patients had higher mean arterial pressure and heart rate. Regarding laboratory results, melioidosis patients had higher blood sugar, potassium, globulin, hemoglobin, lymphocyte, and platelet count. However, they had lower blood lactate, serum creatinine, sodium, and total bilirubin, as shown in Table 2.

Based on the results of multivariable logistic regression analysis, it was observed that melioidosis patients had a lower age (age ≥60 years, OR 0.44, 95% CI 0.25 to 0.77) and a lower frequency of malignancies (OR 0.10, 95% CI 0.04 to 0.22). However, they were predominantly male (OR 4.24, 95% CI 2.29 to 8.20), farmers (OR 3.37, 95% CI 1.84 to 6.26), and had diabetes mellitus (OR 2.65, 95% CI 1.48 to 4.78), with a longer duration of symptoms onset to hospitalization (duration ≥7 days, OR 10.72, 95% CI 5.61 to 21.51) and a higher platelet count (platelet ≥400 x 109/L: OR 3.67, 95% CI 1.34 to 10.24; platelet 200 to 399.9 x109/L: OR 1.60, 95% CI 0.88 to 2.93; platelet <200 x109/L is a reference group) as shown on Table 3.

The clinical prediction model for melioidosis incorporates seven features, all of which are presented in Table 3. Table 3 displays the parameter estimates that are utilized in the calculation of the simple score. The seven features are as follows: age ≥60 years (-2 points), male gender (3 points), duration of onset ≥7 days (5 points), occupation as a farmer (3 points), presence of diabetes mellitus (2 points), presence of malignancy (-5 points),

Table 1. Demographic characteristics of melioidosis and other gram-negative bacteremia patients

Characteristic	All patients (426)	Other Gram-negative bacteremia (294)	Melioidosis (132)	p-value
Age, median (IQR), years	62 (54 to 71)	64 (56 to 74)	58 (51.8 to 64.2)	<0.001*
Male sex, No. (%)	261 (61.3)	157 (53.4)	104 (78.8)	<0.001*
Body mass index, median (IQR), kg/m^2	22.1 (19.5 to 24.3)	21.8 (19.0 to 24.1)	22.6 (20.6 to 24.5)	0.017*
Occupation, No. (%)				
Farmer	123 (28.9)	62 (21.1)	61 (46.2)	<0.001*
Goverment officer	103 (24.2)	72 (24.5)	31 (23.5)	0.919
Company employee	35 (8.2)	28 (9.5)	7 (5.3)	0.202
Self-employed	32 (7.5)	21 (7.1)	11 (8.3)	0.816
Monk	6 (1.4)	2 (3.0)	4 (3.0)	0.077
Unemployed	123 (28.9)	107 (36.4)	16 (12.1)	<0.001*
Charlson comorbidities index, median (IQR), points	4 (2 to 6)	5 (3 to 7)	3 (1.75 to 4)	<0.001*
Comorbidities, No. (%)				
DM	154 (36.2)	79 (26.9)	75 (56.8)	<0.001*
Malignancies	125 (29.3)	115 (39.1)	10 (7.6)	0.001*
Cirrhosis	53 (12.4)	40 (13.6)	13 (9.8)	0.354
CKD stage 3 to 5	49 (11.5)	37 (12.6)	12 (9.1)	0.378
Neurologic diseases	28 (6.6)	23 (7.8)	5 (3.8)	0.179
Chronic pulmonary disease	26 (6.1)	15 (5.1)	11 (8.3)	0.285
CVS diseases	23 (5.4)	17 (5.8)	6 (4.5)	0.771
Connective tissue diseases	12 (2.8)	10 (3.4)	2 (1.5)	0.357
Thalassemia	8 (1.9)	4 (1.4)	4 (3.0)	0.260
AIDS	3 (0.7)	2 (0.7)	1 (0.8)	0.99
Immunocompromised host	51 (12)	34 (11.6)	17 (12.9)	0.822
SOFA, median (IQR), points	3 (2 to 6)	4 (2 to 6)	3 (1 to 5)	<0.001*
APACHE II, median (IQR), points	49.5 (36 to 65)	53 (38 to 66)	43 (30.8 to 57.2)	<0.001*
Steroid use	21 (4.9)	14 (4.8)	7 (5.3)	0.99
Chemotherapy	26 (6.1)	18 (6.1)	8 (6.1)	0.99

AIDS=acquired immunodeficiency syndrome; APACHE=acute physiology, age, chronic health evaluation; CKD=chronic kidney disease; CVS=cardiovascular disease; DM=diabetes mellitus; IQR=interquartile range; SOFA=sequential organ failure assessment

* p<0.05 when compared with other gram-negative bacteremia.

Table 2. Clinical and laboratory characteristics of melioidosis and other gram-negative bactermia patients

Characteristic	All patients (426)	Other Gram-negative bacteremia (294)	Melioidosis (132)	p-value
Chief complaint duration, median (IQR), days	2 (1, 5.75)	1 (1, 3)	7 (3, 14)	<0.001*
Site of infection, No. (%)				
Primary bacteremia	342 (80.3)	263 (89.5)	79 (59.8)	<0.001*
Skin and soft tissues	35 (8.2)	0 (0)	35 (26.5)	<0.001*
Urinary tract infection	21 (4.9)	20 (6.8)	1 (0.8)	0.015*
Respiratory tract infection	17 (4)	11 (3.7)	6 (4.5)	0.901
Intra-abdominal infection	7 (1.6)	1 (0.3)	6 (4.5)	0.004*
Bone and joints	6 (1.4)	0 (0)	6 (4.5)	0.001*
CNS infection	2 (0.5)	0 (0)	2 (1.5)	0.096
Sepsis, No. (%)	304 (81.7)	222 (87.7)	82 (68.9)	<0.001*
Septic shock, No. (%)	26 (6.1)	21 (7.1)	5 (3.8)	0.263
Mechanical ventilation, No. (%)	44 (10.3)	21 (7.1)	23 (17.4)	0.002*
Body temperature, median (IQR), Celsius	38.2 (37.2, 39)	38 (37.2, 38.8)	38.2 (37.3, 39.1)	0.178
Mean arterial pressure, median (IQR), mmHg	80 (73, 90)	78 (72, 87)	85.5 (76, 94.2)	<0.001*
Heart rate, median (IQR), /min	100 (89, 120)	100 (85, 116)	106 (90, 120)	0.009*
Respiratory rate median (IQR), /min	22 (20, 26)	22 (20, 26)	22 (20, 28)	0.724
Blood sugar, median (IQR), mg/dL	158 (122, 242)	140 (116, 207)	224 (150, 311)	<0.001*
Hemoglobin A1C, median (IQR), %	7.9 (6, 11.4)	7.1 (6, 9.8)	8.9 (6.18, 11.6)	0.058
Lactate, median (IQR), mmol/L	2.44 (1.64, 4.18)	2.8 (1.82, 4.57)	2 (1.46, 2.8)	0.001*
Arterial pH, median (IQR)	7.41 (7.32, 7.47)	7.4 (7.34, 7.47)	7.42 (7.29, 7.47)	0.879
PaO2/FiO2, median (IQR)	352 (277, 486)	364 (269, 504)	343 (295, 463)	0.707
PaCO2, median (IQR), mmHg	29 (23.3, 32)	29 (23.2, 33)	30 (23.8, 31.2)	0.870
BUN, median (IQR), mg/dL	18.9 (12.6, 32)	20.5 (13.2, 34)	17.6 (11.8, 26)	0.013*
Creatinine, median (IQR), mg/dL	1.14 (0.8, 1.87)	1.22 (0.8, 2.01)	1.06 (0.78, 1.43)	0.032*
Sodium, median (IQR), mEq/L	136 (132, 139)	136 (132, 139)	134 (130, 137)	<0.001*
Potassium, median (IQR), mEq/L	3.8 (3.4, 4.2)	3.7 (3.4, 4.1)	4 (3.5, 4.3)	<0.001*
Bicarbonate, median (IQR), mEq/L	22.1 (19.5, 24.3)	21.9 (19.4, 24.1)	23.2 (20.0, 25.2)	0.210
Chloride, median (IQR), mEq/L	94.5 (90, 99)	95 (90, 99)	94 (89, 98)	0.148
Albumin, median (IQR), g/dL	2.8 (2.35, 3.3)	2.7 (2.3, 3.2)	2.8 (2.4, 3.4)	0.196
Globulin, median (IQR), g/dL	3.3 (2.8, 3.8)	3.15 (2.7, 3.68)	3.4 (3, 4)	0.001*
Total bilirubin, median (IQR), mg/dL	1.8 (0.7, 4.6)	2.4 (0.8, 6.4)	1 (0.5, 2.2)	<0.001*
ALT, median (IQR), U/L	47 (25.5, 90)	42 (23, 87.5)	54 (33, 91.5)	0.075
AST, median (IQR), U/L	68 (34, 135)	68 (31.8, 142)	68 (36, 130)	0.8696
Alkaline phosphatase, median (IQR), U/L	187 (110, 371)	193 (108, 359)	175 (118, 374)	0.7898
Hemoglobin, median (IQR), g/dL	9.9 (8.4, 11.6)	9.6 (8.1, 11.2)	10.7 (9.17, 12.5)	<0.001*
White blood cell count, median (IQR), x 109/L	13.4 (10, 18.7)	13.8 (10.4, 19.4)	13.1 (8.95, 17.0)	0.067
PMN, median (IQR), %	84 (76, 90.5)	85.2 (76.6, 91.3)	81.6 (74.4, 88.2)	0.007*
Lymphocyte, median (IQR), %	7.3 (3.2, 13.6)	6.4 (2.9, 11.6)	9.05 (5.57, 16.1)	<0.001*
Platelet count, median (IQR), x 109/L	215 (125, 298)	188 (112, 273)	276 (167, 372)	<0.001*

urea nitrogen; IQR=interquartile range

* p<0.05 when compared with other gram-negative bacteremia

and platelet count (x109/L) (200 to 399.9: 1 point, ≥400: 3 points). The simple melioidosis score ranges from a minimum of -7 to a maximum of 16 points.

Model performance and calibration

The simple melioidosis score exhibited an AUC of 0.89 (95% CI 0.86 to 0.93), as indicated in Figure 1. The Hosmer-Lemeshow goodness of fit test yielded p=0.25. A Youden index cut point of ≥5 points resulted in an accuracy of 84.3% (95% CI 80.5 to 87.6), a sensitivity of 78.8%, and a specificity of 86.7%. The calibration curve presented in Figure 2 illustrates that the observed risk and predicted probability are in good agreement for predicting melioidosis. The authors performed a decision curve analysis to evaluate

Table 3. Development of simple melioidosis diagnosis score with the multivariable regression model

Prediction	Parameter estimate+	Simplified score++	Adjusted odds ratio (95%CI)	p-value
Age ≥60 years	-0.823	-2	0.439 (0.247 to 0.772)	0.005
Male	1.445	3	4.243 (2.286 to 8.196)	<0.001
Chief complaint duration ≥7 days	2.372	5	10.72 (5.607 to 21.51)	<0.001
Farmer	1.214	3	3.368 (1.843 to 6.258)	<0.001
Diabetes mellitus	0.973	2	2.646 (1.484 to 4.777)	0.001
Malignancy	-2.314	-5	0.099 (0.040 to 0.221)	<0.001
Platelet count, x109/L				
<200	0	0	1	Ref
200 to 399.9	0.470	1	1.60 (0.877 to 2.932)	0.125
≥400	1.301	3	3.674 (1.341 to 10.24)	0.012
Constant	-2.669	-	-	-

+ Parameter estimates were derived from multivariable logistic regression; ++ Maximum score is 16 points, and the minimum score is -7 points

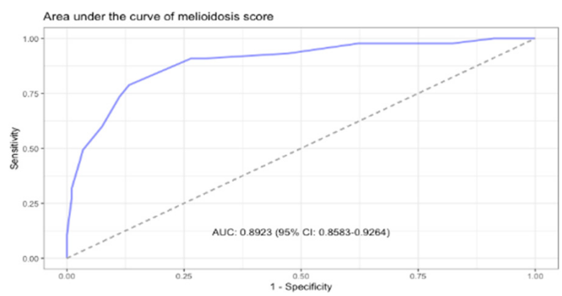

Figure 1. The area under the receiver operating characteristic (ROC) curve for predicting melioidosis.

the clinical utility of our prediction model for predicting the risk of melioidosis. The analysis showed that our model had a higher net benefit than the baseline strategies across a wide range of threshold probabilities, as shown in Figure 3.

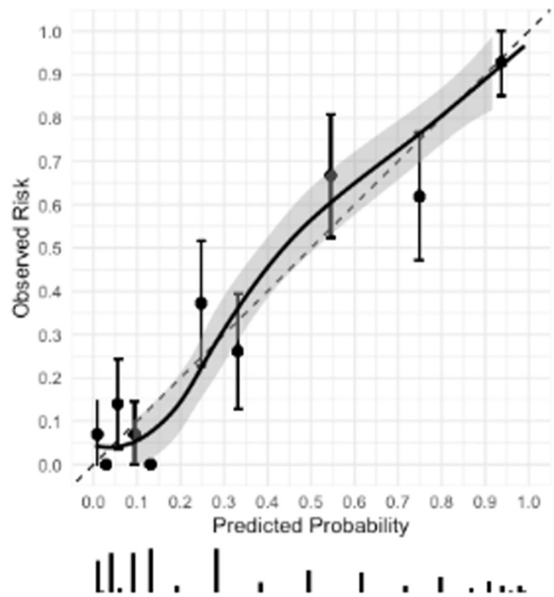

Figure 2. Calibration curve for prediction melioidosis diagnosis. The dashed line on the diagonal respresents the perfect prediction of the ideal model, and the solid line represents the performance of the prediction model. The closer to the diagonal dashed line, the better prediciton effect.

Discussion

The present study identified seven features for predicting melioidosis septicemia. These features included lower age, male gender, longer duration of symptoms onset to hospitalization, occupation as a farmer, presence of diabetes mellitus, absence of cancer, and a higher platelet count. The simple scoring for the diagnosis of melioidosis septicemia demonstrated high predictive performance and clinical utility.

Currently, there is no specific prediction score for the diagnosis of melioidosis. However, studies have identified risk factors associated with melioidosis infection. Some known risk factors for melioidosis include occupational exposure to soil and water, diabetes mellitus, chronic kidney disease, alcohol abuse, and thalassemia[7-12]. In addition, melioidosis is more common in males and older adults[7,10,11]. While these risk factors can help identify individuals who may be at higher risk for melioidosis, they are not specific to the disease and cannot be used as a diagnostic tool.

The median age of our study (58 years) was higher than the average ages of previous studies conducted in Northeastern, Northern, and Eastern parts of Thailand. However, it was similar to reports from the tropical Northern part of Australia, Malaysia, Singapore and India[5,7-10,13,14]. Consistent with this knowledge, most cases were male, which could be due to the fact that males are more often involved in outdoor activities leading to exposure to soil and surface water, compare to females.

Melioidosis is a soil and water-borne infection. The authors found that the proportion of patients with high-exposure occupations, such as farmers and agriculture

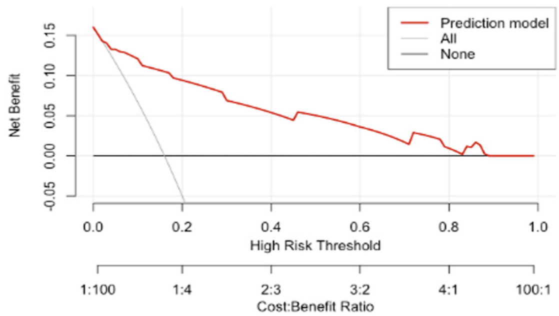

Figure 3. The decision curve compares the net benefits of predicting the probability of melioidosis under three scenarios: perfect prediction model (grey line), no screening (horizontal black solid line), and screening based on a scoring (thick red solid line).

workers, was similar to previous reports in Australia, Northeast Thailand, and Southern Thailand(3,7,11,12,15). This is consistent with the known risk factors for melioidosis, which include occupational exposure to soil and water.

Diabetes mellitus is well established as an important risk factor for melioidosis(6). The most likely mechanism for the predisposition is the impairment of neutrophil functions, including chemotaxis, phagocytosis, and intracellular killing, while the inhibitory effect of insulin on growth of *B. pseudomallei* is minimal.

Cancer was not a significant risk factor for melioidosis infection. This is consistent with other studies that have also failed to find a significant association between cancer and melioidosis. In contrast, our study found that other gram-negative infections were highly associated with cancer. This may be because cancer patients are often immunocompromised, which can make them more susceptible to infections in general. Additionally, cancer patients may undergo treatments such as chemotherapy or radiation therapy, which can further weaken their immune system and increase their risk of infections. Other factors, such as hospitalization or exposure to medical devices, may also play a role in the increased risk of gram-negative infections in cancer patients.

Our study found that platelet count in melioidosis patients was higher than in patients with other gram-negative infections. One possible explanation for this finding is that melioidosis patients may have a less severe form of sepsis compared to patients with other gram-negative infections. The severity of sepsis can be assessed using various scoring systems, such as the Sequential Organ Failure Assessment (SOFA) score and the Acute Physiology and Chronic Health Evaluation (APACHE) score. We also found that patients with other gram-negative infections had higher SOFA and APACHE III scores compared to melioidosis patients, suggesting that patients with other gram-negative infections may have more severe organ dysfunction and a higher risk of mortality.

The authors utilized these features to develop a scoring system for diagnosing melioidosis. The cut-off point of the melioidosis score is ≥5 points (minimum score = -7 and maximum score = 16), with a sensitivity of 0.79 and a specificity of 0.87. The scoring system calculates a total score for each patient, with higher scores indicating a greater likelihood of melioidosis, leading to appropriate evaluation and treatment.

The study's limitations should be considered. Indeed, the fact that the present study was conducted in a single referral university hospital may limit the generalizability of the findings to other settings. The patient population and clinical practices at this hospital may differ from those in other hospitals or regions, which could influence the frequency and severity of melioidosis and other infections. Another limitation is that the study focused solely on bloodstream infections and did not examine other types of melioidosis infections, such as respiratory, skin and soft tissue, or intra-abdominal infections. We did not include SOFA score, APACHE score, or laboratory parameters such as blood sugar level in the predictive model, which might have affected the predictive accuracy. Due to the limited study population, we only evaluated internal validation, which may result in a drop in our prediction score performance when applied to real-world data. Lastly, the retrospective design of this study may have led to missing or incomplete data.

Conclusion

The melioidosis score, based on seven parameters, can be employed by clinicians in real-world practice to predict melioidosis infection. The score may serve as a guide for empirical antimicrobial treatment in the future.

What is already known on this topic?

Melioidosis patients are often experience severe conditions and high mortality rates. An appropriate antibiotic treatment are essential for successful treatment.

What this study adds?

Initiating appropriate empirical antibiotic treatment would result from an early diagnosis based on this score.

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Conflict of interest

The authors declare no conflict of interest.

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