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Low leucocyte, neutrophil and lymphocyte count (tri-low phenotype) in melioidosis: A predictor of early mortality

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ABSTRACT

Introduction: Melioidosis is a bacterial disease caused by *Burkholderia pseudomallei*, a gram-negative bacillus endemic to parts of Asia and Northern Australia. This study aimed to identify the role of total and differential leucocyte count in predicting 48-h mortality in patients with melioidosis.
Methodology: This retrospective cohort study included patients diagnosed with culture-proven melioidosis at Kasturba Medical College between 2017 and 2023. Total leucocyte count (TLC), absolute neutrophil count (ANC), and absolute lymphocytic count (ALC) were classified into low (first quartile), medium (second and third quartile) and high (last quartile). The chi-square test was used to compare each group's early (48-h) mortality.
Results: Of the 170 patients with culture-confirmed melioidosis, 24 patients died within 48 h. The mortality was significantly higher in those with low TLC, ANC and ALC. When all three parameters were found to be low (tri-low phenotype), the specificity in predicting mortality was 93.2 %.
Conclusion: Low TLC, ANC and ALC are significant predictors of mortality among melioidosis patients. There is a need to explore new strategies to improve clinical outcomes among melioidosis patients with tri-low phenotype.

Introduction

Melioidosis, caused by a gram-negative bacterium, *Burkholderia pseudomallei*, is endemic in India, and its incidence is slowly increasing (Birnie et al., 2019). This disease commonly manifests in individuals exposed to soil or surface water, especially in the rainy season. Uncontrolled diabetes, chronic alcohol consumption, and immunosuppression are the main risk factors for the development of melioidosis (Singh et al., 2022). The clinical spectrum of melioidosis is broad, ranging from localised abscesses to septic shock with organ dysfunction (Currie et al., 2021; Currie et al., 2010). In a previous study, we reported high mortality in patients presenting to the emergency department, particularly within the first 48 h (Nisarg et al., 2024). Importantly, initiation of appropriate antibiotics at admission did not appear to improve mortality, suggesting that this subgroup requires more than antibiotics and supportive care (Nisarg et al., 2024). Although the study did not show any impact of the duration of illness at presentation on mortality, the delay in presentation may be linked to higher mortality (Nisarg et al., 2024). It is essential to identify patients at risk of high mortality and develop novel therapeutic strategies that can be utilised in this group of patients. A simple and inexpensive biomarker such as leucocyte count is needed to identify those with a high risk of early mortality and select suitable candidates for these strategies. This study investigates the association between total and differential leucocyte counts and early mortality in patients with melioidosis.

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Methodology

Patients of all ages with melioidosis presenting between 2017 and 2023 to our tertiary care hospital were included in this retrospective cohort study. Melioidosis was defined as the isolation of *B. pseudomallei* from any specimen (blood, sterile fluids, pus, urine, tissue) obtained from a symptomatic patient. It was identified by Matrix-assisted laser desorption and ionisation- time of flight mass spectrometry [bioMérieux Vitek MS (bioMérieux, Marcy-l'Etoile, France) with the Vitek MS research use only database] and confirmed by a specific monoclonal antibody latex agglutination test (Mahidol Oxford Tropical Medicine Research Unit, Bangkok) and Type 3 Secretion System polymerase chain reaction assay (Tellapragada et al., 2017).

Total leucocyte count (TLC), absolute neutrophil count (ANC), absolute lymphocytic count (ALC) and Neutrophil-lymphocyte ratio (NLR) at admission (from a single counter used throughout the period) were obtained from the laboratory information system. Those patients for whom any of these parameters could not be obtained were excluded from the study. Those patients with pre-existing conditions that can lead to cytopenias, such as aplastic anaemia, were excluded. The study's primary outcome was early mortality (mortality within 48 h of presentation to the hospital), whereas the secondary outcome was late mortality (mortality within 28 days of admission). In the South-Indian context, when the family feels that there is futility (due to a rapidly evolving disease and approaching demise) and cannot afford the cost of care, they leave against medical advice. Those patients who left against medical advice before 48 h because of rapidly worsening disease were also included in the mortality if they were likely to die within 48 h from admission.

Median TLC, ANC, ALC and NLR were compared between the patients who died within 48 h and those who did not, using the Mann-Whitney U test. Those variables that were significant were taken for further analysis. The significant variables were each divided into four quartiles. The first quartile was considered as the 'low' group. The last quartile was considered as the 'high' group. The second and third quartiles were combined to form the 'medium' TLC group. The mortality in each group was compared to see if there was a significant difference. The chi-square test was used to compare mortality in each of the groups. A scoring system was created to predict early mortality using the first "low" quartiles of TLC, ANC, and ALC, and a score of 1 was assigned for each parameter. A maximum score of 3 was designated when TLC, ANC and ALC were all categorised as low. A Receiver operating characteristic (ROC) curve was created to predict early mortality.

Results

Of the 172 patients with culture-confirmed melioidosis admitted during this period, two were excluded because they did not have differential counts available. Of the 170 patients, 24 patients died within 48 h, and 49 patients died within 28 days. Two patients left against medical advice in this cohort but were included as mortality. Both these patients were critically ill and left the hospital due to financial constraints and perceived futility of care. The treating team was reasonably sure that the death must have occurred within 48 h of admission. The study investigators felt that excluding these two patients would falsely decrease the true early mortality rates (Fig. 1).

The median TLC, ANC and ALC were significantly lower in those who died within 48 h and those who did not (p-values = 0.027, 0.027 and <0.001, respectively) (Table 1). NLR was not significantly different between the two groups ($p = 0.213$). Lower ALC was also associated with significantly higher mortality at 28 days ($p = 0.001$) (Table 2).

After categorizing TLC, ANC and ALC into low (first quartile), medium (second and third quartile) and high (last quartile), the mortality was significantly higher in those with low TLC, ANC and ALC (Table 3 and Fig. 2).

Using the individual cut-off to classify TLC, ANC, and ALC as low, we created a scoring system where a score of 1 for each parameter was given

Table 1
Comparison of leucocyte parameters in terms of early mortality (at 48 h).

Parameters	Early mortality (n = 24)	Survival (n = 146)	p-value
Total Leucocyte Count) (/μl)	7150 (2800–15,275)	11,600 (8400–15,400)	0.027
Absolute Neutrophil Count (/μl)	4800 (1684–12,792)	8987 (5876–12,388)	0.027
Absolute Lymphocyte count (/μl)	510 (254–817)	1269 (828–1940)	<0.001
Neutrophil Lymphocyte ratio	9.2 (4.6–19.2)	6.8 (4.2–11.7)	0.213

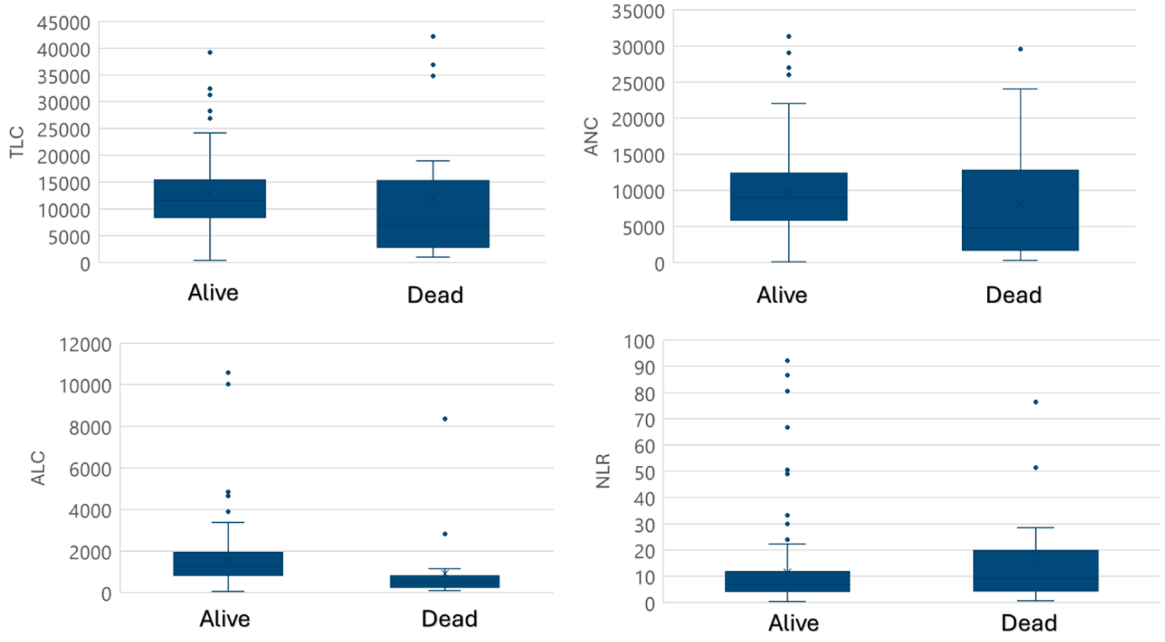

Fig. 1. Comparison of early mortality with leucocyte parameters.

Table 2
Comparison of leucocyte parameters in terms of late mortality (at 28 days).

Parameters	Late mortality (n = 49)	Survival (n = 121)	p-value
Total Leucocyte Count) (/μl)	11,500 (6100–16,100)	11,300 (8300–14,650)	0.583
Absolute Neutrophil Count (/μl)	8807 (3500–13,000)	8802 (5700–11,600)	0.737
Absolute Lymphocyte count (/μl)	844 (377–1400)	1303 (800–1900)	0.001
Neutrophil Lymphocyte ratio	8.4 (4.7–15.1)	6.6 (4–10.8)	0.09

Table 3
Early mortality in low, medium and high total leucocyte count, absolute neutrophil count and absolute lymphocyte count.

Parameter				Early Mortality (n = 24)	p-value
	Category	Number	Definition		
Total Leucocyte Count	Low	49	<7900/μl	14 (58.3 %)	0.001
	Medium	68	7900/μl–15,400/μl	3 (12.5 %)	
	High	53	>15,400/μl	7 (29.2 %)	
Absolute Neutrophil Count	Low	50	<5245/μl	14 (58.3 %)	0.001
	Medium	77	5245/μl–12,387/μl	4 (16.7 %)	
	High	43	>12,387/μl	6 (25 %)	
Absolute Lymphocytic Count	Low	42	<615/μl	14 (58.3 %)	<0.001
	Medium	86	615/μl–1869/μl	8 (33.33 %)	
	High	42	>1869/μl	2 (8.3 %)	

when they were found to be low. With a maximum score of 3, the scoring system had an Area under the curve (AUC) of 0.749 (95 %CI: 0.637–0.861) with a p-value of $p < 0.001$. A score of 1 or above had a sensitivity and specificity of 79.2 % and 60.3 %, respectively. A score of 2 or more was found to have a sensitivity and specificity of 58 % and 79 %, respectively. A score of 3 was found to have sensitivity and specificity of 37.5 % and 93.2 %, respectively. When the scoring system was used to predict late mortality, an AUC of only 0.6 (95 %CI: 0.5–0.7) was found with a p-value of 0.039 (Fig. 3).

Discussion

Patients with septic melioidosis experience high early mortality despite receiving appropriate antimicrobial therapy and supportive care (https, 2024; Mardhiah et al., 2021; Cheng et al., 2003). We investigated the role of TLC, ANC and ALC in predicting mortality and found that the early mortality was highest in the 'low' groups. If a patient was classified into the low group in all three parameters (TLC, ANC and ALC), what we have termed as the 'tri-low' phenotype, the specificity in predicting mortality was very high (93.2 %).

In this study, we did not use standard definitions of leucopenia, neutropenia and lymphopenia, as we anticipated very few patients meeting the criteria of these definitions, thereby decreasing the cut-off's sensitivity for the developed scoring system. We divided the patients into four quartiles based on leukocyte count to investigate whether there is higher mortality at both extremes. It should be noted that each quartile did not have an equal number of patients because of the repetition of the same values. In sepsis, increased mortality can be attributed to both hypo- and hyperimmune responses, as reflected by the association of mortality with both low and high leucocyte counts (https, 2024; Cheng et al., 2003; Finfer et al., 2023; Jenjaroen et al., 2015; Andreu-Ballester et al., 2021). In the study by Hwang et al., the authors divided the sepsis patients into five quintiles based on the neutrophil-to-lymphocyte ratios (Sy et al., 2017). They observed high mortality in the lowest and the highest quintiles. Similarly, in a study on patients with melioidosis, higher mortality rates were observed with both low and high neutrophil counts (Jenjaroen et al., 2015). We observed higher mortality in low leucocyte, neutrophil and lymphocyte counts. Similar to previous studies, we also observed a slightly higher mortality with high leucocyte and neutrophil counts. We focussed on the lower counts as these subgroups' effect was more pronounced.

As any infection progresses, it is often accompanied by immune dysregulation and multi-organ dysfunction syndrome (MODS) (Cao et al., 2023). The immune dysregulation can be in the form of the hyperimmune phenotype, characterised by exaggerated cytokine response or the immunoparalyzed phenotype, where the immune system is exhausted. Differentiating between hyperimmune and immunoparalyzed phenotypes in sepsis is challenging and is a crucial factor in the failure of immunomodulatory strategies (Venet and Monneret, 2018). In melioidosis patients, the tri-low phenotype can be potentially used to identify immune paralysis.

To explore potential immunomodulatory strategies, there is a need

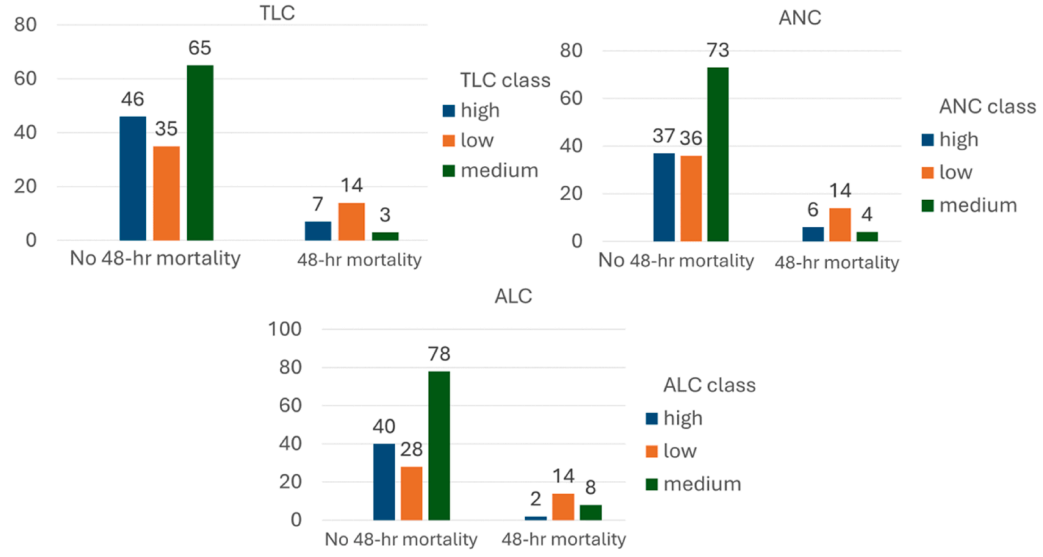

Fig. 2. Comparison of 48-h mortality in patients with low, medium and high total leucocyte count, absolute neutrophil count and absolute lymphocyte count [The number on the Y-axis and the top of the column represents the count of patients in each group (low, medium, high) with mortality or no mortality].

Parameter	Cut-off	Score assigned
Total Leucocyte Count	<7900/µl	1
Absolute Neutrophil Count	<5245/µl	1
Absolute Lymphocytic Count	<615/µl	1

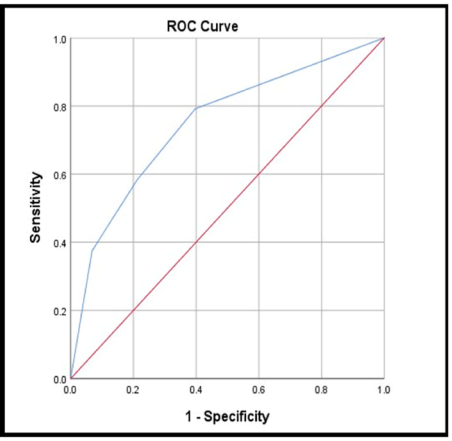

Fig. 3. Details of the scoring system and the Receiver Operating Characteristic Curve of the scoring system to predict early mortality [Area under the curve: 0.749 (95 %CI: 0.637–0.861)].

for simple and inexpensive biomarkers that can guide the selection of appropriate individuals who can benefit. Granulocyte Colony-Stimulating Factor (G-CSF) has previously been studied in septic melioidosis as a promising adjunct to standard treatment (Cheng et al., 2004). However, a subsequent randomised controlled trial did not demonstrate a statistically significant mortality benefit from G-CSF despite observing a delay in mortality among patients who received the drug (Cheng et al., 2007). It is important to note that the study centre faced limited resources, such as a lack of dialysis and mechanical ventilation facilities. This resulted in significantly high all-cause mortality and may have obscured any potential benefits of G-CSF (Cheng et al., 2007). It is also possible that a particular subset of patients that benefitted from G-CSF was missed in the study. Melioidosis patients with a low neutrophil count may be more likely to benefit from adjunctive G-CSF therapy.

Inhibiting the Programmed Cell Death protein-1(PD-1)/ PD-Ligand-1 (PD-L1) pathway may provide a novel approach to enhancing immune responses in patients with melioidosis. *B. pseudomallei* evades the host immune system through various mechanisms, including upregulating PD-1 on lymphocytes and PD-L1 on neutrophils, which impairs protective T-cell responses. PD-1 and PD-L1 blockers, now increasingly indicated for patients with infectious diseases, can reverse immune paralysis and restore immune function (Mariappan et al., 2021; Menon et al., 2020). By blocking the PD-1/PD-L1 pathway, these immune checkpoint inhibitors can rejuvenate T cell function and improve overall immune responses (Buddhisa et al., 2015; Chang et al., 2013). In a study on septic patients with lymphopenia, PD-1 blockers were associated with a faster improvement in SOFA scores, a measure of sepsis severity, without serious adverse events (Hotchkiss et al., 2019). It is possible that the PD-1/PD-L1 blockade can be a promising strategy in melioidosis patients with low lymphocyte counts.

Steroids have been shown to reduce the duration of vasopressor dependency in patients with septic shock without a significant increase in hospital-acquired infections (Venkatesh et al., 2018; Sprung et al., 2008). Using steroids in melioidosis patients can be risky as most patients are either already immunosuppressed or have uncontrolled type 2 diabetes or both. While there might be potential benefits of steroid use in lymphopenic septic melioidosis patients, further studies are needed to evaluate its use, especially in those with high mortality (tri-low phenotype).

Effective management of critically ill patients involves not only optimising antibiotic usage but also addressing immune modulation (Leite and de Lima, 2016; Bunch et al., 2023). Although advancements have been made in antimicrobial usage, immunomodulation remains complex. The heterogeneity of patient populations included in randomised trials often leads to negative results, underscoring the need for individualised approaches applied to different disease phenotypes (Laffey and Kavanagh, 2018). The ``tri-low'' phenotype may represent an immunoparalyzed state in melioidosis, presenting an opportunity to explore immunomodulatory treatments in this subset.

Our study aimed to identify an immunophenotype that could facilitate immunomodulation in septic melioidosis patients. In the treatment of sepsis-related infections, it is essential to address several factors, including immune dysregulation, vascular resuscitation (e.g. coagulopathy, endothelial damage), metabolic resuscitation (e.g. acidosis), and supportive care (including mechanical ventilation, dialysis, fluid management, and transfusions), all of which significantly influence mortality rates. By concentrating exclusively on leucocyte count, we ignored other important parameters, such as platelet count, which can also be considered a part of the immune response and influence patient outcomes. We identified this as a significant limitation of the study. Also, the increase or decrease trends in the counts would have been more helpful in predicting mortality. We selected the admission leucocyte counts for uniformity and ease of comparison and to exclude the impact of agents that might have impacted the counts. Another limitation was the low number of outcome events that precluded us from using additional statistical analyses. Despite these limitations, our research highlights a specific immunological phenotype that may offer opportunities for targeted interventions. Besides, we intended to find a simple and rapid biomarker that can be used in primary care settings.

In conclusion, melioidosis patients with low TLC (<7900/µl), ANC (<5245/µl) and ALC (<615/µl) are at higher risk of mortality. There is a need to explore immunomodulatory strategies to improve clinical outcomes in this subset of patients.

CRediT authorship contribution statement

Nitin Gupta: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Praveen Kumar Tirlangi:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Writing – original draft, Writing – review & editing. **Prithvishree Ravindra:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Writing – original draft, Writing – review & editing. **Rachana Bhat:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Writing – original draft, Writing – review & editing. **Mukund Gupta:** Formal analysis, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Carl Boodman:** Formal analysis, Software, Supervision,

Validation, Visualization, Writing – original draft, Writing – review & editing. **Adil Rashid:** Formal analysis, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Chiranjay Mukhopadhyay:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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