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## Clinical Utility of Platelet Count as a Prognostic Marker for Melioidosis

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**Abstract.** Thrombocytopenia predicts mortality in patients with melioidosis in Thailand. We analyzed platelet counts in two large cohorts of melioidosis patients in tropical northern Australia to assess utility in a different clinical setting. Admission platelet counts were compared between subgroups of patients with different clinical outcomes. Patients with more severe disease (indicated by bacteremia, septic shock, and death) had significantly lower platelet counts than those with less severe disease. Logistic regression analysis was carried out for potential predictors of mortality among various clinical parameters, and platelet count was shown to be an independent predictor of mortality. Furthermore, in patients critically ill with melioidosis, an increasing platelet count after admission was associated with a significantly greater chance of survival. However, given that most patients with severe disease still had platelet counts within the normal range, platelet count is not a useful biomarker for predicting the severity of melioidosis in a clinical context.

### INTRODUCTION

The role of platelets in the complex pathophysiology of sepsis remains to be fully elucidated, but thrombocytopenia is commonly seen in severe sepsis.<sup>1,2</sup> In Thailand, thrombocytopenia was common in diabetic patients with severe melioidosis and correlated with mortality.<sup>3</sup> Further studies from Thailand have now confirmed that thrombocytopenia was present in 31% of all melioidosis patients and predicted mortality.<sup>4</sup> In addition, in a murine experimental model of melioidosis, platelets were suggested to have a protective role in both innate immunity and vascular integrity.<sup>4</sup> The implications of these studies for both clinical assessment and potential future adjunctive therapies in melioidosis remain unclear.

The Darwin Prospective Melioidosis Study (DPMS) from tropical northern Australia commenced in 1989, and an earlier analysis of outcomes showed a number of variables that were independently predictive of mortality; pneumonia at primary presentation, diabetes, hazardous alcohol intake, renal impairment, age at admission, lymphocyte count, serum urea, serum bilirubin, and serum bicarbonate.<sup>5</sup> Platelet count was not included in that analysis, and we have now analyzed platelet counts in the Darwin cohort, with additional data analyzed from a cohort of melioidosis patients from Cairns in tropical North Queensland.

### METHODS

For both cohorts, the case definition was all patients with melioidosis as confirmed by culture of *Burkholderia pseudomallei* from any clinical specimen. The DPMS database and parameter definitions were as previously described.<sup>5,6</sup> Platelet counts on or as near as possible to the date of admission were collected from April 1999 until February 2017. In addition, for patients who developed septic shock, a second platelet count was recorded as close as possible to the date of death or discharge from intensive care. For these patients the change in platelet count was calculated as the admission platelet count subtracted from this second platelet count.

Statistical tests were performed using STATA15 for Windows (StataCorp LP, College Station, TX). Platelet counts were compared across subgroups of patients based on clinical outcomes: bacteremia, septic shock, and death. The two-sample Wilcoxon rank-sum test was used to determine statistical significance. Box plots were generated to compare the distribution of platelet counts between subgroups. The proportion of patients with thrombocytopenia (platelet count less than  $150 \times 10^9/L$ ) in each subgroup was also calculated. For patients who developed septic shock, the change in platelet count between admission and intensive care discharge or death was compared between patients who recovered and those who died from their illness.

Logistic regression analysis was carried out for potential predictors of mortality. Clinical parameters previously shown to be independently associated with mortality<sup>5</sup> (diabetes, hazardous alcohol intake, renal impairment, age at admission, lymphocyte count, serum urea, serum bilirubin, and serum bicarbonate) were extracted from the DPMS database. Bivariate logistic regression analysis was conducted for each of these variables in addition to admission platelet count. Variables found to be significant (using a significance level of 0.05) on bivariate analysis were confirmed on multivariate analysis by stepwise exclusion of nonsignificant variables from the multivariate model.

To increase the external validity from the DPMS findings, demographic and clinical information and admission platelet counts were collected from all patients admitted with melioidosis to Cairns Hospital between January 1998 and April 2017,<sup>7</sup> with admission platelet counts compared between subgroups of patients based on clinical outcomes.

The studies were approved by the Human Research Ethics Committees of the Northern Territory Department of Health and Menzies School of Health Research (HREC 02/38) and the Far North Queensland Human Research Ethics Committee (HREC/15/QCH/46-977).

### RESULTS

Admission platelet counts were available for 758/791 (96%) patients admitted to the Royal Darwin Hospital with culture-confirmed melioidosis between April 1999 and February 2017. Of these 758 patients, 447 (59%) were male, the median age was 50 years (range 7 months–97 years), and 371 (49%) were

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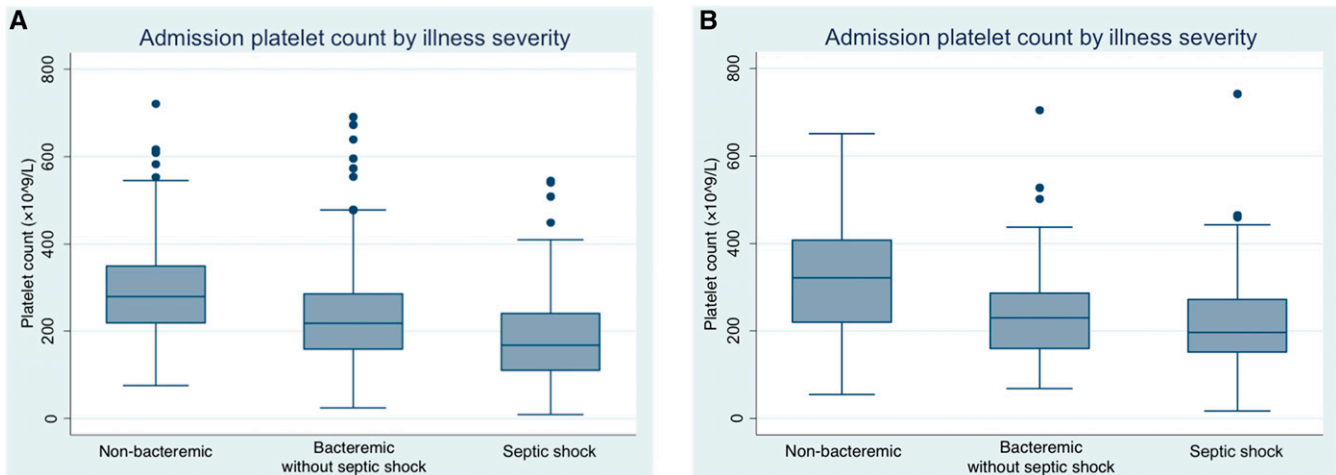


FIGURE 1. (A) Admission platelet counts for 758 patients in the Darwin Prospective Melioidosis Study, grouped by illness severity. (B) Admission platelet counts for 218 patients at Cairns Hospital, grouped by illness severity. This figure appears in color at [www.ajtmh.org](http://www.ajtmh.org).

of Aboriginal or Torres Strait Islander origin. Four hundred and fifty-seven (60%) had bacteremia, 167 (22%) developed septic shock, and 66 (9%) died.

The mean admission platelet count was  $242 \times 10^9/L$ , with a standard deviation of  $113 \times 10^9/L$ . Patients with bacteremia and those with septic shock had significantly lower platelet counts, with mean platelet counts  $211 \times 10^9/L$  (95% CI:  $201\text{--}221 \times 10^9/L$ ) and  $181 \times 10^9/L$  (95% CI:  $166\text{--}196 \times 10^9/L$ ), respectively, when compared with those patients without bacteremia or without septic shock,  $290 \times 10^9/L$  (95% CI:  $278\text{--}302 \times 10^9/L$ ) and  $259 \times 10^9/L$  (95% CI:  $250\text{--}268 \times 10^9/L$ ), respectively (both  $P < 0.001$ ). Figure 1A shows the decreasing trend in platelet count with increasing disease severity.

Patients who died from melioidosis had significantly lower admission platelet counts than patients who survived, with mean platelet counts  $187 \times 10^9/L$  (95% CI:  $161\text{--}213 \times 10^9/L$ ) and  $247 \times 10^9/L$  (95% CI:  $239\text{--}255 \times 10^9/L$ ), respectively ( $P = 0.001$ ). Of those patients who died, 41/66 (62%) had a normal platelet count ( $> 150 \times 10^9/L$ ) on admission, 14/66 (21%) had a count less than  $100 \times 10^9/L$  and 5/66 (8%) had a count less than  $50 \times 10^9/L$ .

In patients with septic shock, a rise in platelet count over the course of intensive care admission was associated with survival. 40/78 (84%) patients whose platelet counts increased survived compared with 74/88 (51%) patients whose platelet counts decreased. Among the patients who survived, the mean rise in platelet count was  $56 \times 10^9/L$  (95% CI:  $30\text{--}83 \times 10^9/L$ ) compared with patients who died, who had a mean decrease in platelet count of  $51 \times 10^9/L$  (95% CI: decrease of  $75\text{--}27 \times 10^9/L$ ) ( $P < 0.001$ ) (Figure 2).

In the bivariate model, the following factors were significantly associated with mortality; platelets, age, hazardous alcohol use, serum bilirubin, serum urea (log transformation), and serum bicarbonate (data not shown). Using stepwise removal of variables, platelets, age, bilirubin, urea, and bicarbonate were each independently associated with mortality in the multivariate model (data not shown). The multivariate model indicated that reduced platelet count is an independent predictor of mortality in melioidosis patients, with a  $50 \times 10^9/L$  increase in platelet count associated with a reduction in mortality by a factor of 22%.

Admission platelet counts were available for 218/225 (97%) patients with culture-confirmed melioidosis identified at Cairns Hospital between January 1998 and April 2017. One hundred and sixty-three (74%) were male, the average age was 50 years, and 129 (59%) were of Aboriginal or Torres Strait Islander origin. One hundred and sixty-five (76%) had bacteremia, 68 (31%) developed septic shock, and 30 (14%) died.

The mean admission platelet count was  $231 \times 10^9/L$ , with a standard deviation of  $121 \times 10^9/L$ . The same correlation between platelet count and severity of illness was seen in this second data set (Figure 1B). Patients with bacteremia and those with septic shock had significantly lower platelet counts, with mean platelet counts  $227 \times 10^9/L$  (95% CI:  $210\text{--}245 \times 10^9/L$ ) and  $217 \times 10^9/L$  (95% CI:  $187\text{--}246 \times 10^9/L$ ), respectively, when compared with those patients without bacteremia or without septic shock,  $323 \times 10^9/L$  (95% CI:  $289\text{--}356 \times 10^9/L$ ) ( $P < 0.001$ ) and  $266 \times 10^9/L$  (95% CI:  $237\text{--}295 \times 10^9/L$ ) ( $P = 0.002$ ), respectively.

Again, patients who died had lower platelet counts than patients who survived, with mean platelet counts  $216 \times 10^9/L$

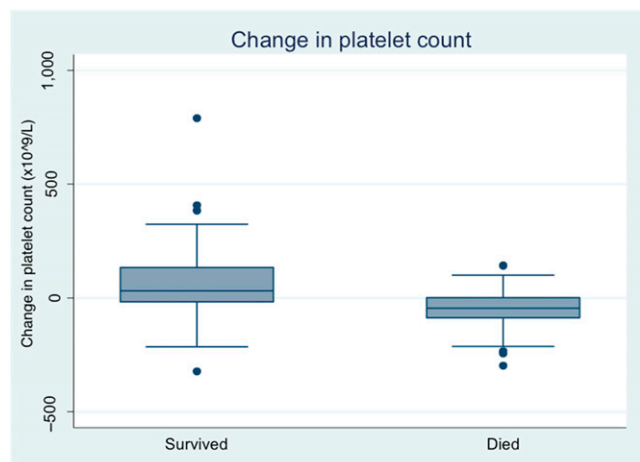


FIGURE 2. Change in platelet count during intensive care admission for patients with septic shock in the Darwin Prospective Melioidosis Study, grouped according to survival. This figure appears in color at [www.ajtmh.org](http://www.ajtmh.org).

(95% CI: 165–267 × 10<sup>9</sup>/L) and 256 × 10<sup>9</sup>/L (95% CI: 239–273 × 10<sup>9</sup>/L), respectively; however, this was not significant ( $P = 0.11$ ). Of those patients who died, 20/30 (67%) had a normal admission platelet count (> 150 × 10<sup>9</sup>/L), 6/30 (20%) had a count less than 100 × 10<sup>9</sup>/L and 4/30 (13%) had a count less than 50 × 10<sup>9</sup>/L.

## DISCUSSION

The results from this large number of patients confirm that admission platelet count is an independent predictor of severity of and mortality due to melioidosis in Australia, as previously shown in Thailand. Furthermore, in patients critically ill with melioidosis, an increasing platelet count after admission is associated with a significantly greater chance of survival. Nevertheless, there is substantial overlap in the range of platelet counts shown for each illness severity cohort (Figure 1). For instance, of the fatal cases, 41/66 (62%) in Darwin and 20/30 (67%) in Cairns had a normal platelet count at presentation. This cautions against using platelet counts to predict severity or outcomes in patients with melioidosis. This is similar to a prior analysis of C-reactive protein (CRP) from the DPMS, which found that the admission CRP levels may be normal or only mildly elevated in patients with severe sepsis and in subsequently fatal cases.<sup>8</sup>

The development of thrombocytopenia in sepsis is secondary to various mechanisms that result in platelet activation, sequestration, and destruction.<sup>9</sup> The pathogenesis of melioidosis includes extensive abnormalities in the coagulation system such as excess Von Willebrand factor and deficiency of ADAMTS13 (A Disintegrin And Metalloprotease with a ThromboSpondin type 1 motif, member 13).<sup>3</sup> These findings have been described in other patients with severe sepsis.<sup>10</sup> However, correlation analysis suggested that these two markers of endothelial stimulation were not the primary drivers of thrombocytopenia in the melioidosis patients in Thailand.<sup>3</sup> Other possible causes of the thrombocytopenia in melioidosis include disseminated intravascular coagulation (DIC) and hemophagocytosis.<sup>11</sup> Although these possibilities have not been systematically studied in the DPMS, DIC was not found to be overtly evident in the Thai cohort.<sup>3</sup>

Understanding of the role of platelets in the host response against bacterial pathogens in general and against *B. pseudomallei* specifically is evolving, in particular the interactions of platelets with neutrophils and the innate immune system.<sup>12,13</sup> The murine model of melioidosis showed that mice depleted of platelets had decreased survival and increased bacterial numbers in the lungs.<sup>4</sup> However, in that study, platelet depletion did not directly influence growth of *B. pseudomallei*, but did impair early neutrophil recruitment to the lungs, with the conclusion that platelets play a protective role in both innate immunity and in vascular integrity. Taken together, data from the cohorts from Australia and Thailand show that thrombocytopenia is associated with a more disturbed host response in melioidosis. Nevertheless, although the animal studies suggest that reduced platelet counts may lead directly to adverse outcomes per se, it remains possible that the thrombocytopenia seen in severe melioidosis in humans is predominantly secondary to and a reflection of the severity of disease.

In conclusion, admission platelet count is a predictor of severity and mortality in melioidosis when analyzing a large cohort of patients, but for individual patients, it is not a useful

biomarker to predict outcomes, with more than 60% of those patients dying from melioidosis still having an admission platelet count in the normal range.

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## REFERENCES

1. Claushuis TA, 2016. Thrombocytopenia is associated with a dysregulated host response in critically ill sepsis patients. *Blood* 127: 3062–3072.
2. Vieira-de-Abreu A, Campbell RA, Weyrich AS, Zimmerman GA, 2012. Platelets: versatile effector cells in hemostasis, inflammation, and the immune continuum. *Semin Immunopathol* 34: 5–30.
3. Birnie E, Koh GCKW, Löwenberg EC, Meijers JCM, Maude RR, Day NPJ, Peacock SJ, van der Poll T, Wiersinga WJ, 2017. Increased Von Willebrand factor, decreased ADAMTS13 and thrombocytopenia in melioidosis. *PLoS Negl Trop Dis* 11: e0005468.
4. Birnie E et al., 2018. Thrombocytopenia impairs host defense against *Burkholderia pseudomallei* (melioidosis). *J Infect Dis*, doi: 10.1093/infdis/jiy541 (Epub ahead of print).
5. Cheng AC, Jacups SP, Anstey NM, Currie BJ, 2003. A proposed scoring system for predicting mortality in melioidosis. *Trans R Soc Trop Med Hyg* 97: 577–581.
6. Currie BJ, Ward L, Cheng AC, 2010. The epidemiology and clinical spectrum of melioidosis: 540 cases from the 20 year Darwin prospective study. *PLoS Negl Trop Dis* 4: e900.
7. Stewart JD, Smith S, Binotto E, McBride WJ, Currie BJ, Hanson J, 2017. The epidemiology and clinical features of melioidosis in Far North Queensland: implications for patient management. *PLoS Negl Trop Dis* 11: e0005411.
8. Cheng AC, O'Brien M, Jacups SP, Anstey NM, Currie BJ, 2004. C-reactive protein in the diagnosis of melioidosis. *Am J Trop Med Hyg* 70: 580–582.
9. Venkata C, Kashyap R, Farmer JC, Afessa B, 2013. Thrombocytopenia in adult patients with sepsis: incidence, risk factors, and its association with clinical outcome. *J Intensive Care* 1: 9.
10. Kremer Hovinga JA, Zeerleder S, Kessler P, Romani de Wit T, van Mourik JA, Hack CE, ten Cate H, Reitsma PH, Wuillemin WA, Lämmle B, 2007. ADAMTS-13, von Willebrand factor and related parameters in severe sepsis and septic shock. *J Thromb Haemost* 5: 2284–2290.
11. Smith S, Munas AM, Hanson J, 2018. Haemophagocytic lymphohistiocytosis complicating melioidosis. *Am J Trop Med Hyg* 99: 557–558.
12. de Stoppelaar SF, van 't Veer C, Claushuis TA, Albersen BJ, Roelofs JJ, van der Poll T, 2014. Thrombocytopenia impairs host defense in gram-negative pneumonia-derived sepsis in mice. *Blood* 124: 3781–3790.
13. Wiersinga WJ, Virk HS, Torres AG, Currie BJ, Peacock SJ, Dance DAB, Limmathurotsakul D, 2018. Melioidosis. *Nat Rev Dis Primers* 4: 17107.