Platelet count:

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Platelet count: a predictor of sepsis and mortality in severe burns?

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Abstract

Background: Platelet cells, or thrombocytes, have additional roles to haemostasis. Post-burn injury, platelet counts drop to a nadir at day 2-5 then rise to a peak between days 10-18. The nadir has previously been associated with mortality but there is currently no thorough investigation of its potential to predict sepsis in adults. The primary objective of this study is to assess whether platelet count can predict survival and sepsis in adults with severe burn injuries. **Methods and Findings:** A retrospective cohort analysis of platelet count and other blood parameters in 145 burn patients with a TBSA greater than 20%. AUROC analysis revealed that the platelet count and rBaux score together produce moderate discrimination for survival at less than 24 hours post-injury (AUROC = 0.848, 95%CI 0.765-0.930). Platelet count at day 3 combined with TBSA has a modest association with sepsis (AUROC = 0.779, 95%CI 0.697-0.862). Multivariable Cox regression analysis revealed platelet peak was the strongest predictor of mortality. **Conclusions**: A reduced peak platelet count is a strong predictor of 50-day mortality. Platelet count nadir may have some association with sepsis.

Introduction

Platelets are known traditionally for their essential roles in haemostasis and thrombosis. However, their non-haemostatic roles as sentinels of the innate immune system during infection and inflammation are becoming increasingly recognised[1–3]. Several large clinical studies conducted in intensive care units suggest that thrombocytopenia is predictive of mortality and multiple organ failure during sepsis[4–6]. However, in burn injury, the diagnosis of sepsis is often more difficult due to a profound systemic inflammatory response obscuring the classical signs and diagnostic criteria. Intriguingly, platelet counts post-burn injury tend to follow a distinct pattern; falling to a nadir at day 2-5, then rising to a peak value at day 10-18. This has been investigated within animal models, case reports[7–9], and a number of larger scale studies[10–12]. A number of these studies have compared platelet counts and mortality[10,11,13]. More recently, Marck et al. investigated platelet counts within a large heterogeneous group (N = 244) of adult and paediatric burns patients, where 80% of the cohort had burns covering less than 29% total body surface area (TBSA). They compared both the nadir and peak values with mortality[14]. Both the mean nadir and peak platelet counts were significantly lower in both septic and non-surviving patients with lower peak counts predicting 50 day mortality ($p < 0.05$). However, Marck et al had very few septic patients in their cohort; hence, there has not been a proportional hazards model applied to an adult dataset of burns patients to investigate platelet count and sepsis.

In this retrospective study of 145 patients with severe burn injuries (≥20% TBSA) we investigate whether the classical pattern of post-burn platelet counts are able to predict outcomes. In addition, we also examine if other routinely measured haematological parameters are helpful to the clinician in their assessment of the patient.

Materials and Methods

Patient Cohort

This retrospective cohort study was conducted from January 2007 to May 2015. All burn patients were screened for eligibility. Table 1 shows the inclusion and exclusion criteria for the study. Clinical data were collected from the electronic patient record (EPR) and UK International Burn Injury Database (IBID) including: age at injury; gender; body mass index (BMI); length of stay in total (LOS) and in intensive care episodes (LOS ICU); mechanism of injury; inhalation injury status and severity; TBSA%; sepsis and mortality. Each patient was assessed for the presence of sepsis through appraisal of the EPR, paper records and observation charts.

Table 1. Inclusion and exclusion criteria.

Routine Haematological and Pathology Measurements

Routine haematological parameters were extracted from the EPR for 50 days post-burn injury. These included: platelet count; white blood cell counts including the differential of lymphocytes and neutrophils and C-Reactive protein (CRP). All cellular parameters were measured in the routine cellular pathology laboratories at Queen Elizabeth Hospital Birmingham (QEHB) using a Beckman Coulter UniCel DxH 800 Cellular Analysis System from 2010 - 2015, and with a Beckman Coulter LH750 from 2007-2010. Both analysers use impedance based analysis for platelets with similar accuracy and precision[15,16]. Quality control was ensured by regular measurement of internal and external quality control samples.

Clinical Definitions

The primary outcomes were in-hospital 50-day mortality and incidence of sepsis. Sepsis was defined as a patient meeting a score of 3 or more using the 2007 American Burn Association criteria plus a temporally relevant positive microbiological culture result, (±5 days from the ABA indicated sepsis)[17]. Severity of injury was reported using the revised-Baux (rBaux)

score, defined by Osler et al[18]. This was preferred over other mortality scoring systems such as the Abbreviated Burn Severity Index (ABSI) as previous diagnostic test accuracy studies show it has greater accuracy in predicting mortality in severe burns[19,20]. Thrombocytopenia was defined as a platelet count of less than $150x10^9$/L, and thrombocytosis as a platelet count of greater than $400x10^9$/L[21]. The neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) were also calculated from routine parameters. Inhalational injury was defined as the presence of carbonaceous deposits, erythema, oedema, bronchorrhea or obstruction observed with or without the aid of bronchoscopy. Severity of inhalational injury was divided into mild, moderate or severe: Mild was defined as minor/patchy areas of erythema and carbonaceous deposits in the proximal or distal bronchi; Moderate as erythema with carbonaceous deposits, bronchorrhea with or without compromise of the bronchi; and severe was defined as any of the following: strong inflammatory response with friability, copious carbonaceous deposits, bronchorrhea, or bronchial obstruction.

The nadir platelet count was defined as the lowest value between days 2-5. The peak platelet count was the highest value observed between days 11-17. These values are based on previous figures from the literature and experimental models[7–12,14].

Statistical Methods

Variables were assessed for normality both graphically, using Q-Q plots, and quantitatively using the Shapiro-Wilk test. The non-normal data are described by a median value with the Inter Quartile Range (IQR). Normal (Gaussian) distributed data are represented with the means and 95% confidence intervals. The Chi-squared test was used to test for significance between categorical variables. For continuous non-Normally distributed variables the Kruskal–Wallis Rank Sum Test or Mann-Whitney U test (if only 2 groups) were used to test for significance. For Normal continuous variables, the one-way ANOVA or Student's t-test (if only 2 groups) were implemented. All tests were two-tailed. Longitudinal modelling of haematological parameters by group (both survival and sepsis) was performed using linear

mixed models to account for the correlation structure imposed by the within-patient repeated-measures data. Graphs of model fitted values were produced with the shaded envelope denoting the 95% confidence intervals. The area under the receiver operator curves (AUROCs) for each parameter were calculated for days 0, 1, 3, 7, 14, 21 and 28 post-burn injury using logistic regression models. These models were adjusted for confounding due to severity of injury through the inclusion of the rBaux score. The outcomes for this analysis were survival and sepsis. All haematological variables were studied. Time to event analysis was conducted using Cox Regression. These models were adjusted for peak thrombocyte count and rBaux score with univariate analyses also carried out for the nadir thrombocyte count. Significance was set at the $p < 0.05$ level. Analyses were performed using the R statistical package (R version 3.3.1)[22]. All graphs were produced using R with the ggplot2 package[23]. The demographics table (table 2) was created using the tableone package[24].

Results

Patient Demographics

A total of 3,975 patients with burns were admitted to the Birmingham adult burns centre at QEHB between 2007 and 2015. After applying inclusion and exclusion criteria, a final study cohort of 145 patients remained (Figure 1). The final demographics of the cohort are displayed in table 2. There were a greater proportion of male patients (59.3%) and the most common mechanism of injury was flame. The average burn size was 30%, with a mean rBaux score of 87.74. Half of the patients had inhalation injuries with 61.1% of those being moderate to severe. The observed mortality rate for the cohort was 24.8% and 41.4% of patients experienced at least one episode of sepsis. Univariate analyses showed some significant associations between variables and the outcomes of sepsis and survival. As expected, survival was significantly lower in the sepsis group. The presence of inhalation injury and LOS were significantly different between septic and non-septic patients. For both

sepsis and survival, significant differences were found in: TBSA, ABSI and rBaux scores, and ICU admission.

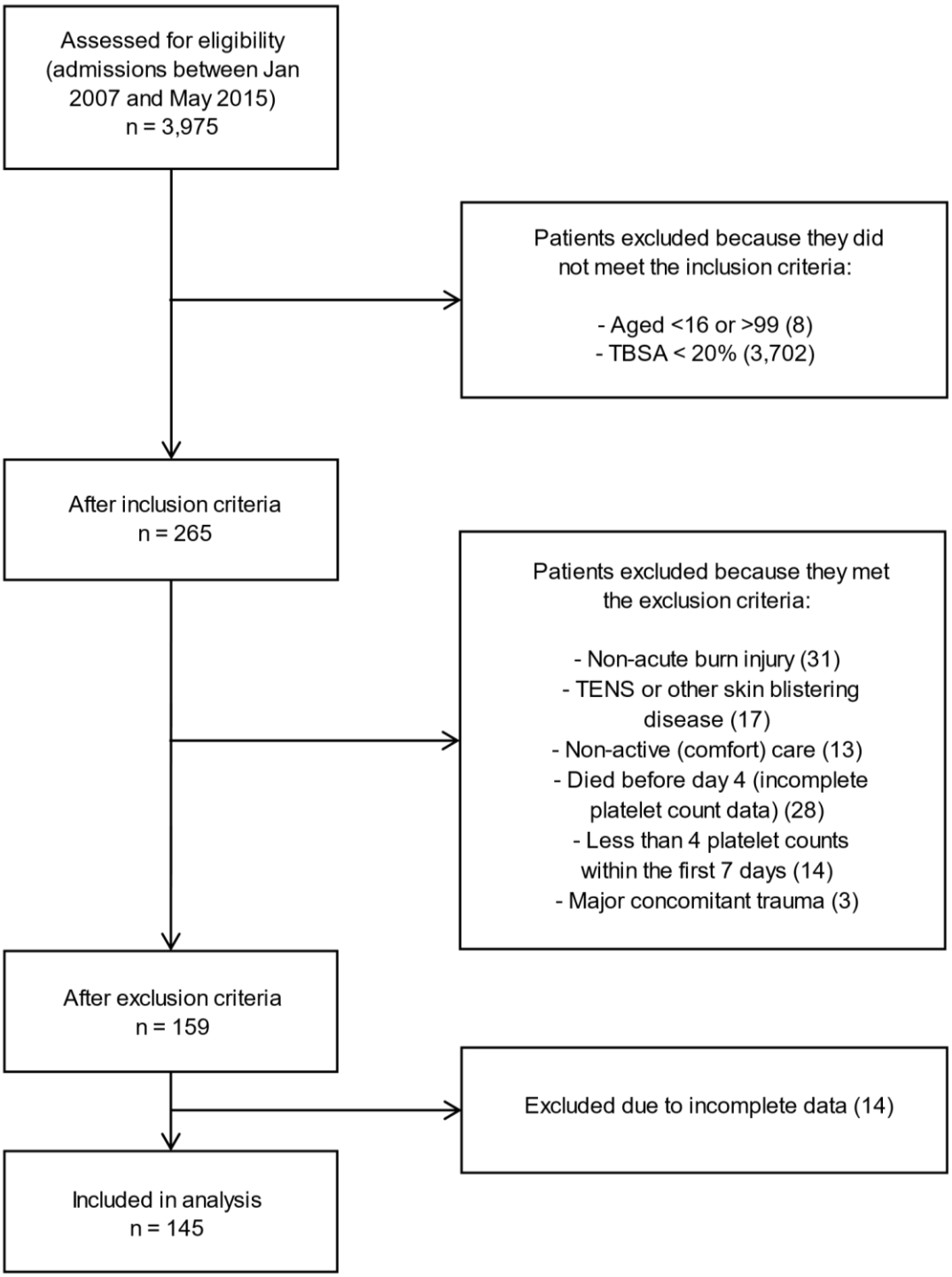

Figure 1. Participant flowchart showing application of exclusion and inclusion criteria.

Table 2. Demographics of study participants. BMI=body mass index; TBSA=total body surface area; FT/DD=full thickness burn ABSI=abbreviated burn severity index; rBaux=revised baux score; ICU=intensive care unit. *Missing data is due to death or discharge at the time of platelet peak count. ns (not shown) p > 0.05, * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001. Square brackets denote the IQR where the median value is displayed and round brackets denote the SD where the mean value is displayed.

Platelet count trajectories stratified by mortality or sepsis

The time course of platelet counts are shown in Figure 2 and depict a classical thrombocytopenic nadir which is between days 2-4 with a peak of thrombocytosis on days 11-17. Analysis of when each patient's individual platelet nadir occurs shows that, on average, the nadir occurs on day 3. 57 (39.3%). Patients had their lowest platelet count on day 2, and 41 (28.3%) had their lowest platelet count on day 3. Interestingly, high platelet counts continue until day 50 post-injury without any indication of decline. This may be artefact due to the decreased frequency of platelet count results at later time-points, where the number of observations range between 114 and 128 across days 2 to 4 and between 77 and 99 across days 11 to 17. However, this difference is not discernible from Figure 2.

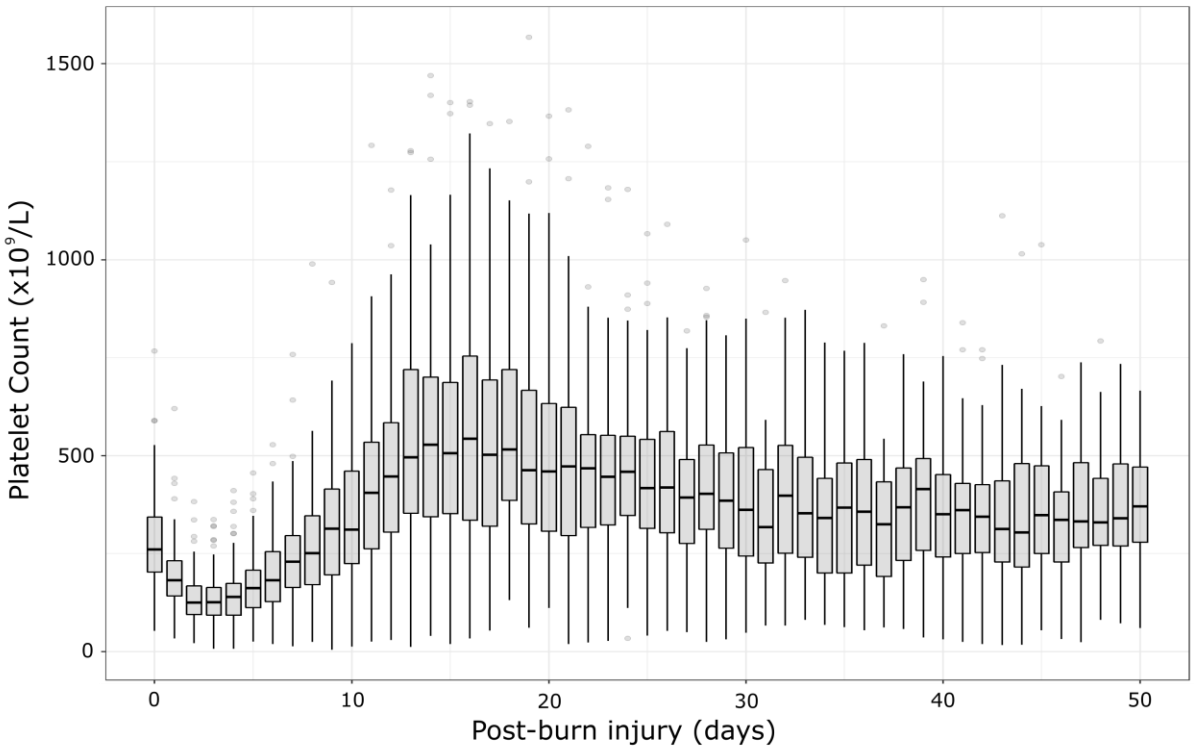

Figure 2. The observed platelet counts for the total cohort of severe burns over 50 days of admission. A nadir at days 2-4 is observed and a peak at days 13-14. The platelet counts are tightly distributed at the nadir but there is wider variability in the data at the peak. Grey points show outlier values.

To help account for the variability between patients in their patterns of platelet count, which is observable in Figure 2, a linear mixed effects modelling framework was applied to the data which included random uncorrelated effects for patient and day. The output of which can be seen in Figure 3.

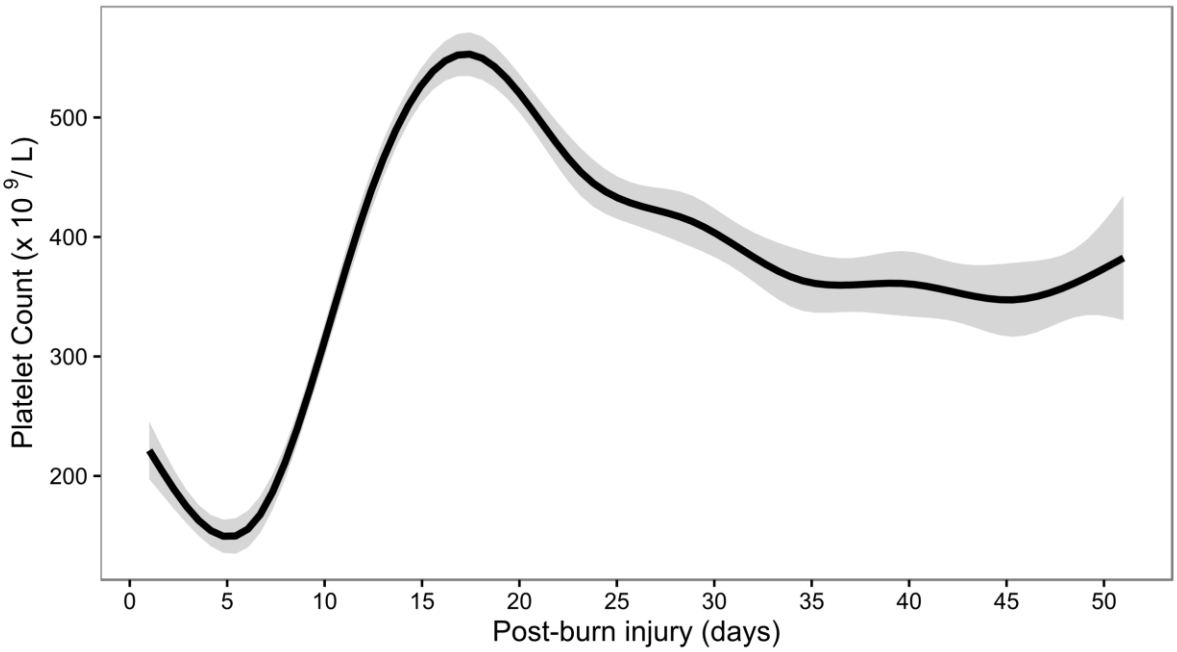

Figure 3. Platelet counts over time of the cohort after application of the linear mixed effects model which helps to account for individual variability across the cohort. Shaded areas represent 95% confidence intervals.

The platelet counts were subsequently stratified by mortality (Figure 4). Survivors, for the most part, displayed a much higher platelet count at the nadir with a significantly greater platelet count at all stages post-day 5. These survivors also exhibited a significantly higher peak of thrombocytosis at day 16 compared with non-survivors. The survivors were still in range of thrombocytosis even at 50-days post-injury. On average, non-survivors did not display thrombocytosis at any given moment within the 50 days post-injury.

When stratifying the cohort by sepsis (Figure 5) the sepsis group reached lower platelet count values at the nadir with marginal overlapping of confidence intervals. Similarly, to the groups stratified by survival, patients with sepsis exhibit a significantly lower platelet count peak at 15-20 days post-injury.

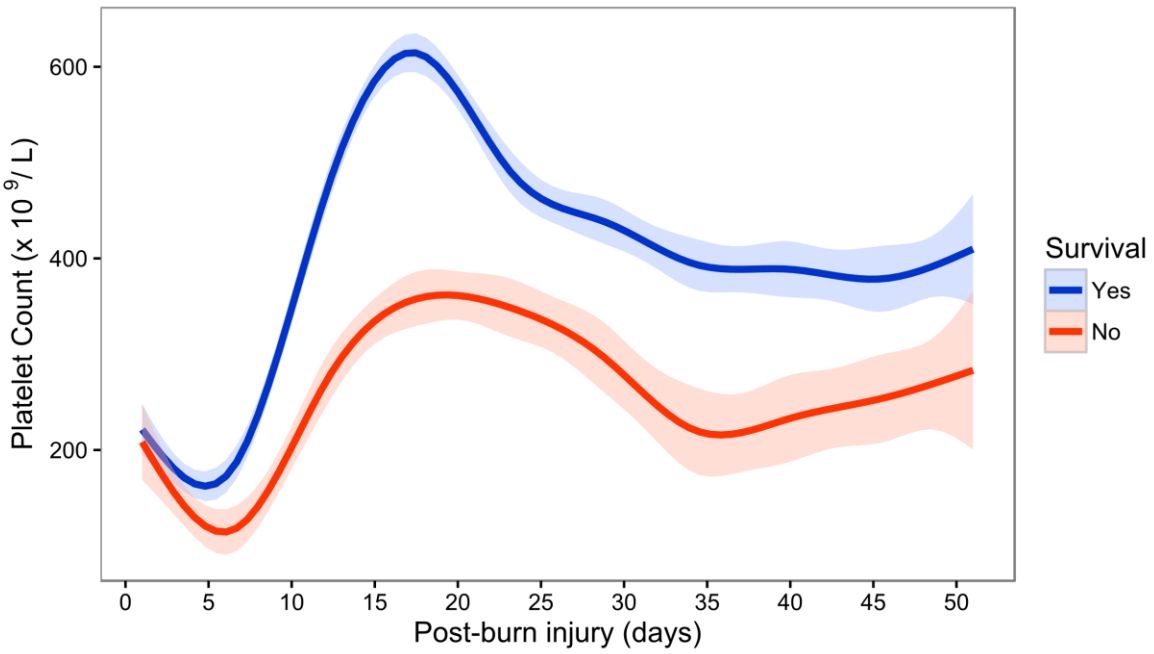

Figure 4. Platelet count stratified by survival. Platelet counts stratified by survival show a significant difference between groups at the nadir and at the peak.

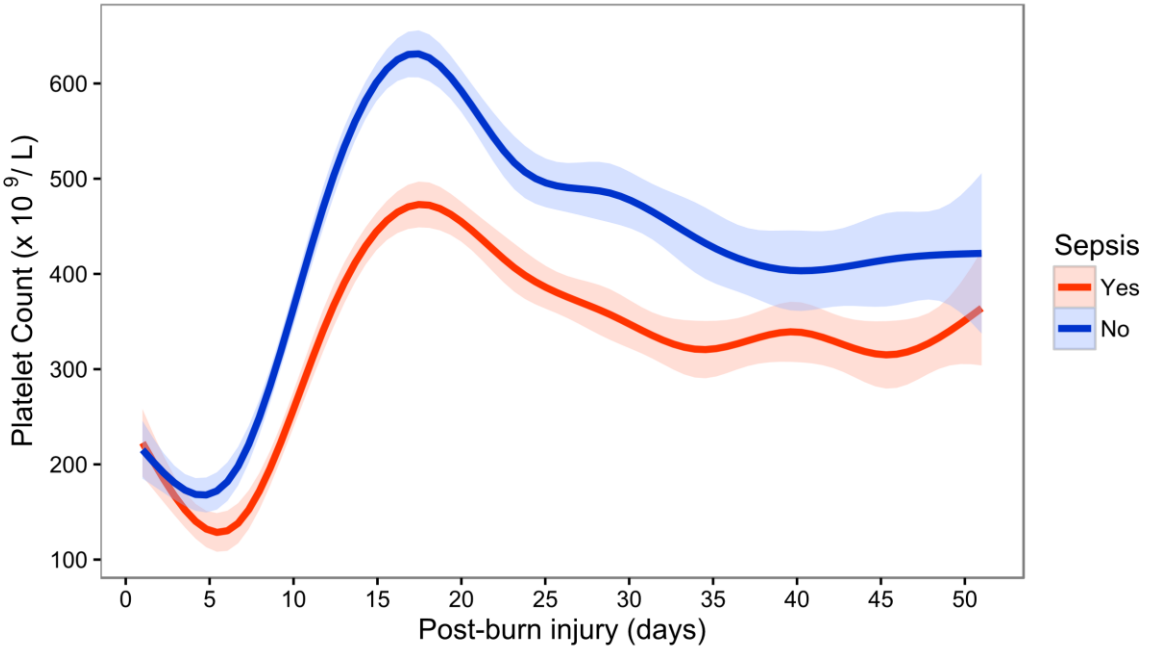

Figure 5. Platelet count stratified by sepsis. Platelet counts stratified by sepsis show a borderline non-significant difference at the nadir, but a significant difference at the peak.

Daily models

Daily logistic regression models were conducted for the nadir (days 2-4) and peak (days 11-17) to investigate the relationship of platelet count on survival and sepsis. Analyses were conducted firstly with platelet count alone as a predictor, then with rBaux score added to the model to adjust for burn injury burden.

Table 3 shows the model outcomes for survival. Survival odds ratios are significant from day 2 post-burn in the nadir period and during all of the peak platelet count period even when adjusted for rBaux score.

Table 3. Daily odds ratios for survival from logistic regression analysis for days 2-4 (platelet count nadir) and days 11-17 (platelet count peak).

The model for sepsis is shown in Table 4. At day 3 post-injury there is a significant result even after adjustment with rBaux score suggesting that on day 3 platelet count may have some relation, and hence prediction, for sepsis. There are also significant results in the peak platelet count range in the univariate analysis.

Table 4. Daily odds ratios for sepsis from logistic regression analysis for days 2-4 (platelet count nadir) and days 11-17 (platelet count peak).

Time to event analysis

Using a time to event analysis (Cox regression) the patients were censored separately for survival and sepsis. Table 5 shows the summarized results from this analysis.

Table 5. Cox regression analysis summarized into a table. Two multivariable models are summarized for each outcome (survival and sepsis): rBaux (a value composed from age, TBSA and inhalation injury) and platelet count (peak or nadir) were included. rBaux was included to correct for severity of injury. Continuous variables where categorised arbitrarily to allow the analysis to occur, hence the values for hazard radio correspond to: rBaux (per 10 points), peak platelet count (per 50x109/L), nadir platelet count (per 50x109/L).

Peak platelet count appears to be related to survival (HR=0.813 (95% CI 0.756-0.874)) but the nadir shows no significant relation (p=0.077). Neither the peak platelet count does not appear to be associated with the hazard of developing sepsis. However, TBSA does have an influence on the multivariable model (p<0.0001), with a 5 percentage point increase in TBSA corresponding to an 18% increase in the hazard of having sepsis. However, even when

adjusted for rBaux the nadir platelet count still shows some relation to sepsis (HR=0.750 (95% CI 0.574-0.979).

AUROC analysis

Area under the receiver operator curve analysis revealed that the platelet count alone shows poor discriminatory power for survival post-burn injury (Day 0, AUROC=0.534, 95%CI 0.387-0.68). However, when combined with the rBaux score there is moderate discriminatory power at less than 24 hours post-injury (AUROC=0.848, 95%CI 0.765-0.93).

In contrast platelet counts throughout the time course had limited power to discriminate between septic and non–septic patients even when combined with the rBaux score (Day 0, AUROC=0.742, 95%CI 0.648-0.835). Interestingly, the predictive power appears stronger when combined with TBSA% rather than rBaux. On days 0, 3 and 14 the AUROC was 0.756 (95%CI 0.662-0.85), 0.779 (95% CI 0.697-0.862) and 0.776 (95% CI 0.676,0.876) respectively showing poor to moderate discriminatory power for predicting sepsis.

Other variables

The graphical representation of the data for NLR, PLR, CRP, and white blood cell counts including neutrophils and lymphocyte counts did not indicate any differences between sepsis and survival groups (data not shown).

Discussion

In this single centre retrospective study of a relatively large cohort of patients with severe burns we have, first of all, re-affirmed the classical pattern of platelet counts post-burn injury. Thrombocytopenia usually occurs with a nadir between days 2 and 5 followed by a peak of thrombocytosis at around day 11-17[7–12]. This early thrombocytopenia could be caused by any number of mechanisms including: haemodilution by resuscitation fluids; platelet activation with subsequent peripheral consumption; or by depressed bone marrow production.

It is remarkably difficult to discern to what degree haemodilution affects platelet count post-burn injury. It is possible there is some effect, however studies investigating fluid replacement and platelet count have shown that low platelet count persists after fluid therapy has been stopped[25,26].

Hence, it is reasonable to suggest that platelets are being consumed within the burn wound as a result of destruction of the dermal vasculature and subsequent microthrombi formation. These microthrombi form by 24-48 hours and so this may coincide with the nadir[27,28]. It is also well documented that the permeability of surrounding vessels increases along with development of widespread vascular hyper-permeability, and this may lead to increased activation of platelets through interaction with tissue factor on the sub-endothelium and activated clotting factors, leading to subsequent aggregation and consumption. Activated platelets may interact with circulating neutrophils and monocytes, potentiating their ability to extravasate into the sites of injury and affecting the platelet peripheral count [1,3].

Bone marrow suppression as an explanation is less likely. Hampson et al showed that neutrophil and immature granulocyte counts are elevated significantly within 24 hours of injury[29]. Hence, there is a response profile suggesting active bone marrow post-burn injury. Previous autopsy studies in severe burns support this assertion as thrombocytopenia has been shown to have no association with fewer bone marrow megakaryocytes[30].

There are various other factors that may affect platelet count. Drugs such as heparin can cause a thrombocytopenia (Heparin Induced Thrombocytopenia (HIT)) but this is typically later than we have seen in our cohort of patients, starting 5-10 days after the use of heparin and hence is unlikely to contribute dramatically, if at all, to our observations[31]. Observations in published case reports have also suggested that in some patients piperacillin-tazobactam, a commonly prescribed antibiotic, can cause thrombocytopenia but these cases are very rare[32,33].

The peak in platelet count for burns patients may be explained by an elevation of circulating Thrombopoeitin (TPO) levels following a fall in overall platelet mass early post-injury. This would stimulate platelet production from the bone marrow and may explain the rebound thrombocytosis that is seen in our cohort. This may also be exacerbated by inflammatory cytokines (e.g IL-6) during the SIRS response post injury.

In our cohort there is an statistically significant difference in the platelet counts between survivors and non-survivors in days 3-4 and indeed also in the peak platelet count, even when corrected for the severity of injury using the rBaux score (OR=0.187 (95% CI 1.11-3.15) and OR=0.175 (95% CI 1.10-2.80) respectively) (**Error! Reference source not found.**). Indeed, this is also apparent from the Cox regression analysis (HR=0.813 (95% CI 0.756-0.874)). From previous burns literature, platelet count does appear to have some relationship with mortality. Wang et al studied massive burns (>70% TBSA) in 102 adults, and found that severe thrombocytopenia (platelet count < $20x10^9$/L) was an independent predictor of mortality ($p < 0.05$)[11]. However, this is quite a substantial thrombocytopenia and such a substantial drop in platelet count is not frequently observed. Guo et al have also demonstrated that a reduction in platelet count of greater than 65% from baseline is predictive of 30-day mortality in burns patients ($p = 0.028$)[10]. It may be possible that the bone marrow response to the initial platelet count drop is different in survivors and non-survivors. This could be due to an enhanced inflammatory response in these individuals stimulating bone marrow activity. Hence, measurement of both TPO and IL-6 levels over time might also be very informative of the status of the megakaryocyte/platelet axis.

There is also a distinct difference between peak platelet count in septic patients compared to non-septic in the daily model analysis on days 11-17, but this difference is only found on days 11 and 12 when combined with rBaux to help correct for disease burden. This is not apparent in the multivariable Cox regression analysis (p=0.445). The effect may be explained due to a reduced platelet lifespan. Pathogenic E.coli and S.aureus have been shown to induce apoptotic mechanisms in platelets, through the degradation of Bcl-x_L an

essential mediator of survival in platelets[34]. In addition, peptidoglycan a major constituent of gram positive bacterial cell walls, has been shown to induce mitochondrial depolarisation and caspase 3 activation, leading to platelet apoptosis[35]. Hence there are numerous mechanisms to suggest a reduced platelet life span in sepsis that may explain the reduced platelet peak observed in septic patients.

The platelet nadir also appears to have some association with sepsis. The Cox regression analysis shows significant values for platelet nadir with sepsis as the outcome (HR=0.750 (95% CI 0.574-0.979)), though data from the daily models adjusted with rBaux suggests this effect is predominantly on day 3 (OR=0.58 (95% CI 0.39-0.85)). However, the AUROC data suggests that this is a poor to moderate predictor. The mechanisms behind this are largely unknown however as discussed earlier, platelets have an important role in immunity. A lower platelet count could lead to a compromised immune response to infection and increase susceptibility to sepsis[36,37]. This is more likely than the converse, primarily due to the early occurrence of the nadir; sepsis is more likely to develop later post-burn injury[38].

Our data shows that both NLR and PLR values do not vary significantly across the time course between the sepsis and survival outcome groups studied. It was therefore not surprising that they showed poor discriminatory power for these outcomes as assessed by AUROC. This contrasts with the findings in the non-burn critical care literature for predicting mortality, sepsis and length of hospitalisation. In one prospective cohort study NLR was shown by multivariable Cox regression to predict in-hospital and 6-month mortality to a reasonable degree (HR=1.63 (1.110-2.415) and 1.58 (1.136-2.213) respectively)[39]. NLR has also been shown to predict mortality in septic patients admitted to critical care (HR=1.043 (1.012–1.083))[40]. PLR has been shown to be associated with mortality and length of stay in critically ill diabetic ketoacidosis patients[41]. This is perhaps another example of the differences in pathophysiology between burn injury and other critical illnesses and the importance of studying burn injury as a discrete entity.

The Beckman Coulter analysers used during this study also measure platelet counts by the Coulter principle (or impedance analysis). There have been reported difficulties with the measurement of platelet counts in burns patients through impedance. This is due to the formation of circulating microspherocytes from the uncontrolled destruction of red blood cells (RBC) during the initial insult of thermal injury[42,43]. It has been previously shown that these RBC derived fragments can potentially interfere with impedance counts as they tend towards the same size range as platelets[42]. This could therefore produce spuriously elevated results and affect the statistical analysis of platelet counts in this and other studies. However, we now feel that this is unlikely due to our recent data directly quantifying these fragments along with 3 different platelet counts (including impedance and fluorescence measurements) post-injury. The results suggest that this interference effect is only significant immediately at day 1 post-injury (Dinsdale et al, 2017. Manuscript submitted).

Diagnosis of sepsis is challenging in patients with severe burn injury because the systemic inflammatory response can mask the classical diagnostic criteria. A limitation of this study, and the other retrospective studies in this area, is in accurately identifying the occurrence of sepsis using clinical criteria. In this study, we used the ABA 2007 Consensus sepsis trigger criteria as these are widely used and burns specific. In 2016, new definitions for sepsis and septic shock were developed and published by a task force from the Society for Critical Care Medicine (SCCM) and the European Society of Intensive Care Medicine (ESICM)[44]. The task force recommends the use of the Sequential Organ Failure Assessment (SOFA) score in ICU patients and the quick-SOFA (qSOFA) score in ward based or emergency department patients. This criterion has not yet been applied in a burns population and warrants evaluation of its discriminatory performance in this setting before it can be applied to the evaluation of potential laboratory diagnostic markers.

Many of the variables we have measured are quantitative laboratory based values and these values are measured less regularly towards the end of a patient's hospital episode. This is demonstrated by the broader confidence intervals towards day 50 post-injury indicating

lower precision in the graphs of the model based fitted values. Additionally, there are no children included in our sample of adult major burns. This was to remove any confounding effects from different platelet kinetic responses, but a disadvantage is that the results may not be generalizable to the paediatric population. Inherently the study design is also problematic when determining causality. Considering this, it is important to highlight that we are establishing the discriminatory power of these haematological parameters and not whether there is a causal link to the outcome of interest.

Conclusions

In conclusion, we have confirmed the kinetics of platelet counts in a large adult cohort of severe burns. With the exclusion of small burns (<20% TBSA) and children, we have removed potential confounders from different kinetic profiles. Platelet count and rBaux score together produce moderate discriminatory power for survival at less than 24hrs post-injury. Additionally, the platelet count at the nadir combined with TBSA has a modest association with sepsis. It was peak platelet count that showed strong predictive power for mortality when in a multivariable model with TBSA, age, rBaux score in the Cox regression model.

In concert with clinical variables and a larger biomarker panel, platelet count may have diagnostic utility and aid the earlier diagnosis of sepsis in patients with severe burns. It appears peak platelet count has an association with mortality, further investigation should focus on why this might be. Together, these findings with future work may highlight patients with a more significant systemic inflammatory response that need tailored care to prevent and monitor for sepsis. Investigation into the mechanism of these platelet kinetics would be valuable for the understanding of physiology following burn injury.

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Tables

Included	Excluded
• Aged 16-99 • Total body surface area percentage (TBSA%) is greater than or equal to 20% • At least one platelet count within 48 hours of injury • A minimum of 4 platelet counts within the first 7 days of admission	• Non-acute burn injury • Diagnosed with platelet disorders. • Patients diagnosed with skin blistering conditions (such as TENS) • Chemical burn injury • Patients admitted for comfort care (where a decision is made within the first 24 hours) • Incomplete data or unable to obtain medical notes

Table 1. Inclusion and exclusion criteria.

	Overall	Sepsis			Survival		
		Yes	No		Yes	No	
N	145	60	85		109	36	
Gender male **(%)**	86 (59.3)	31 (51.7)	55 (64.7)		66 (60.6)	20 (55.6)	
Age	39.00 [28.00, 53.00]	39.00 [30.75, 49.00]	39.00 [28.00, 54.00]		36.00 [28.00, 47.00]	49.00 [37.75, 65.00]	**
BMI	25.86 [22.00, 28.65]	25.39 [22.49, 28.41]	26.12 [23.44, 29.24]		25.39 [22.89, 28.24]	27.44 [24.01, 29.39]	
Mechanism of injury (%)							
Contact	3 (2.1)	0 (0.0)	3 (3.5)		3 (2.8)	0 (0.0)	
Electrical	7 (4.8)	2 (3.3)	5 (5.9)		6 (5.5)	1 (2.8)	
Flame	109 (75.2)	50 (83.3)	59 (69.4)		77 (70.6)	32 (88.9)	
Flash	7 (4.8)	2 (3.3)	5 (5.9)		6 (5.5)	1 (2.8)	
Mixed	6 (4.1)	3 (5)	3 (3.5)		6 (5.5)	0 (0.0)	
Scald	13 (9.0)	3 (5.0)	10 (11.8)		11 (10.1)	2 (5.6)	
TBSA	30.00 [23.00, 48.50]	45.50 [30.00, 59.25]	25.00 [22.00, 31.50]	**	28.00 [22.00, 43.00]	45.50 [30.00, 55.75]	**
FT/DD%	15.00 [4.00, 33.50]	24.75 [10.75, 50.50]	10.00 [2.00, 23.50]	**	10.00 [2.00, 24.00]	33.75 [20.19, 50.50]	**
ABSI	8.00 [7.00, 10.00]	10.00 [9.00, 11.00]	7.00 [6.00, 9.00]	**	8.00 [6.00, 9.00]	10.00 [9.00, 11.00]	**
rBaux score	87.74 (25.24)	100.33 (23.22)	78.85 (22.81)	**	80.72 (22.94)	108.99 (19.51)	**
Inhalation (%)	72 (49.7)	40 (66.7)	32 (37.6)	**	50 (45.9)	22 (61.1)	
Inhalation severity (%)							
Mild	28 (38.9)	11 (27.5)	17 (53.1)		24 (48.0)	4 (18.2)	
Moderate	25 (34.7)	15 (37.5)	10 (31.2)		15 (30.0)	10 (45.5)	
Severe	19 (26.4)	14 (35.0)	5 (15.6)		11 (22.0)	8 (36.4)	
Nadir platelet count (x 10^9 /L)	114.00 [82.00, 149.00]	96.50 [71.75, 125.75]	126.00 [88.00, 164.00]	*	122.00 [88.00, 163.00]	85.00 [68.50, 99.50]	**
Peak platelet	662.68	578.24	719.34	*	722.63	418.08	**

count (x 10^9 /L)	(283.11)	(301.63)	(256.71)		(261.58)	(235.02)	
LOS	34.00 [21.00, 56.00]	45.50 [25.00, 76.25]	28.00 [19.00, 44.00]	**	39.00 [22.00, 57.00]	25.00 [11.75, 35.50]	**
ICU admission (%)	97 (66.9)	57 (95.0)	40 (47.1)	***	64 (58.7)	33 (91.7)	***
ICU LOS	19.00 [7.50, 30.00]	22.01 [9.25, 34.75]	17.00 [7.00, 25.00]		22.01 [8.25, 34.75]	15.00 [7.00, 25.00]	
Survived (%)	109 (75.2)	38 (63.3)	71 (83.5)	*			
Septic (%)	60 (41.4)				38 (34.9)	22 (61.1)	*

Table 2. Demographics of study participants. BMI=body mass index; TBSA=total body surface area; FT/DD=full thickness burn ABSI=abbreviated burn severity index; rBaux=revised baux score; ICU=intensive care unit. *Missing data is due to death or discharge at the time of platelet peak count. ns (not shown) $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$. Square brackets denote the IQR where the median value is displayed and round brackets denote the SD where the mean value is displayed.

		Univariate Analysis			Adjusted for rBaux		
	Day	Survival OR	95% CI	p-value	Survival OR	95% CI	p-value
Nadir	2	1.28	(0.88, 1.88)	0.1999	1.25	(0.83, 1.89)	0.279
	3	2.20	(1.37, 3.52)	0.0010	1.87	(1.11, 3.15)	0.018
	4	2.21	(1.42, 3.45)	0.0005	1.75	(1.10, 2.80)	0.019
Peak	11	1.30	(1.11, 1.53)	0.0010	1.22	(1.04, 1.44)	0.018
	12	1.30	(1.13, 1.50)	0.0004	1.21	(1.04, 1.41)	0.016
	13	1.35	(1.16, 1.57)	0.0001	1.28	(1.08, 1.51)	0.004
	14	1.29	(1.12, 1.48)	0.0004	1.24	(1.07, 1.44)	0.005
	15	1.34	(1.14, 1.56)	0.0003	1.29	(1.08, 1.53)	0.004
	16	1.24	(1.09, 1.41)	0.0011	1.21	(1.05, 1.39)	0.008
	17	1.20	(1.06, 1.35)	0.0038	1.17	(1.02, 1.34)	0.030

Table 3. Daily odds ratios for survival from logistic regression analysis for days 2-4 (platelet count nadir) and days 11-17 (platelet count peak).

		Univariate Analysis			Adjusted for rBaux		
	Day	Sepsis OR	95% CI	p-value	Sepsis OR	95% CI	p-value
	2	0.83	(0.61, 1.13)	0.2428	0.86	(0.61, 1.19)	0.359
Nadir	3	0.52	(0.36, 0.75)	0.0005	0.58	(0.39, 0.85)	0.005
	4	0.71	(0.53, 0.95)	0.0230	0.88	(0.64, 1.20)	0.406
	11	0.83	(0.74, 0.93)	0.0018	0.87	(0.77, 0.98)	0.024
	12	0.84	(0.75, 0.93)	0.0011	0.88	(0.79, 0.98)	0.025
	13	0.91	(0.84, 0.99)	0.0220	0.95	(0.87, 1.03)	0.211
Peak	14	0.90	(0.82, 0.98)	0.0175	0.92	(0.84, 1.01)	0.081
	15	0.91	(0.84, 0.99)	0.0318	0.93	(0.86, 1.02)	0.113
	16	0.92	(0.85, 1.00)	0.0464	0.95	(0.87, 1.03)	0.190
	17	0.88	(0.81, 0.97)	0.0085	0.91	(0.82, 1.00)	0.059

Table 4. Daily odds ratios for sepsis from logistic regression analysis for days 2-4 (platelet count nadir) and days 11-17 (platelet count peak).

			Hazard Ratio	95% CI	p-value
Survival					
Model 1	rBaux		1.124	(0.963, 1.311)	0.137
	Peak platelet count		0.813	(0.756, 0.874)	< 0.0001
Model 2	rBaux		1.251	(1.085, 1.442)	0.002
	Nadir platelet count		0.601	(0.410, 0.881)	0.077
Sepsis					
Model 3	rBaux		1.223	(1.094, 1.366)	0.0004
	Peak platelet count		0.983	(0.941, 1.027)	0.445
Model 4	rBaux		1.186	(1.066, 1.320)	0.002
	Nadir platelet count		0.750	(0.574, 0.979)	0.035

Table 5. Cox regression analysis summarized into a table. Two multivariable models are summarized for each outcome (survival and sepsis): rBaux (a value composed from age, TBSA and inhalation injury) and platelet count (peak or nadir) were included. rBaux was included to correct for severity of injury. Continuous variables where categorised arbitrarily to allow the analysis to occur, hence the values for hazard radio correspond to: rBaux (per 10 points), peak platelet count (per 50x109/L), nadir platelet count (per 50x109/L).

Legends for Illustrations

Figure 1. Participant flowchart showing application of exclusion and inclusion criteria.

REQUIRES COLOUR Figure 2. The observed platelet counts for the total cohort of severe burns over 50 days of admission. A nadir at days 2-4 is observed and a peak at days 13-14. The platelet counts are tightly distributed at the nadir but there is wider variability in the data at the peak. Grey points show outlier values.

Figure 3. Platelet counts over time of the cohort after application of the linear mixed effects model which helps to account for individual variability across the cohort. Shaded areas represent 95% confidence intervals

REQUIRES COLOUR Figure 4. Platelet count stratified by survival. Platelet counts stratified by survival show a significant difference between groups at the nadir and at the peak.

REQUIRES COLOUR Figure 5. Platelet count stratified by sepsis. Platelet counts stratified by sepsis show a borderline non-significant difference at the nadir, but a significant difference at the peak.