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Platelet count:

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

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12 **Keywords:** Sepsis, Burn management, Platelet counts, Mortality

13 **Abstract**

14 **Background:** Platelet cells, or thrombocytes, have additional roles to haemostasis. Post-
15 burn injury, platelet counts drop to a nadir at day 2-5 then rise to a peak between days 10-
16 18. The nadir has previously been associated with mortality but there is currently no
17 thorough investigation of its potential to predict sepsis in adults. The primary objective of this
18 study is to assess whether platelet count can predict survival and sepsis in adults with
19 severe burn injuries. **Methods and Findings:** A retrospective cohort analysis of platelet
20 count and other blood parameters in 145 burn patients with a TBSA greater than 20%.
21 AUROC analysis revealed that the platelet count and rBaux score together produce
22 moderate discrimination for survival at less than 24 hours post-injury (AUROC = 0.848,
23 95%CI 0.765-0.930). Platelet count at day 3 combined with TBSA has a modest association
24 with sepsis (AUROC = 0.779, 95%CI 0.697-0.862). Multivariable Cox regression analysis
25 revealed platelet peak was the strongest predictor of mortality. **Conclusions:** A reduced
26 peak platelet count is a strong predictor of 50-day mortality. Platelet count nadir may have
27 some association with sepsis.

28 **Introduction**

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

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

51 **Materials and Methods**

52 ***Patient Cohort***

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

61 **Table 1. Inclusion and exclusion criteria.**

62 ***Routine Haematological and Pathology Measurements***

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

72 ***Clinical Definitions***

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

77 score, defined by Osler et al[18]. This was preferred over other mortality scoring systems
78 such as the Abbreviated Burn Severity Index (ABSI) as previous diagnostic test accuracy
79 studies show it has greater accuracy in predicting mortality in severe burns[19,20].
80 Thrombocytopenia was defined as a platelet count of less than $150 \times 10^9/L$, and
81 thrombocytosis as a platelet count of greater than $400 \times 10^9/L$ [21]. The neutrophil-lymphocyte
82 ratio (NLR) and platelet-lymphocyte ratio (PLR) were also calculated from routine
83 parameters. Inhalational injury was defined as the presence of carbonaceous deposits,
84 erythema, oedema, bronchorrhea or obstruction observed with or without the aid of
85 bronchoscopy. Severity of inhalational injury was divided into mild, moderate or severe: Mild
86 was defined as minor/patchy areas of erythema and carbonaceous deposits in the proximal
87 or distal bronchi; Moderate as erythema with carbonaceous deposits, bronchorrhea with or
88 without compromise of the bronchi; and severe was defined as any of the following: strong
89 inflammatory response with friability, copious carbonaceous deposits, bronchorrhea, or
90 bronchial obstruction.

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

94 ***Statistical Methods***

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

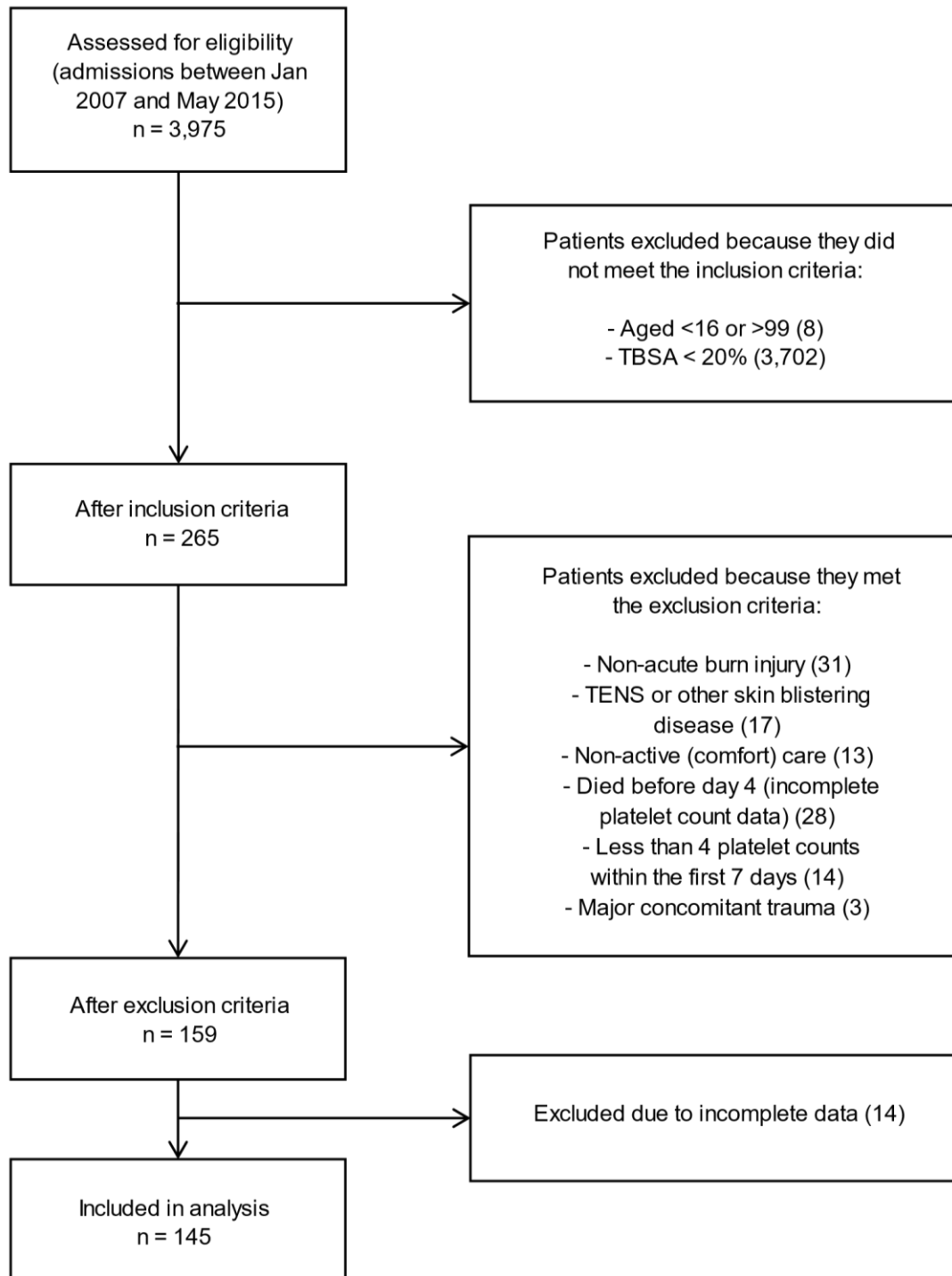
104 mixed models to account for the correlation structure imposed by the within-patient
105 repeated-measures data. Graphs of model fitted values were produced with the shaded
106 envelope denoting the 95% confidence intervals. The area under the receiver operator
107 curves (AUROCs) for each parameter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

117 **Results**

118 ***Patient Demographics***

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

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



132

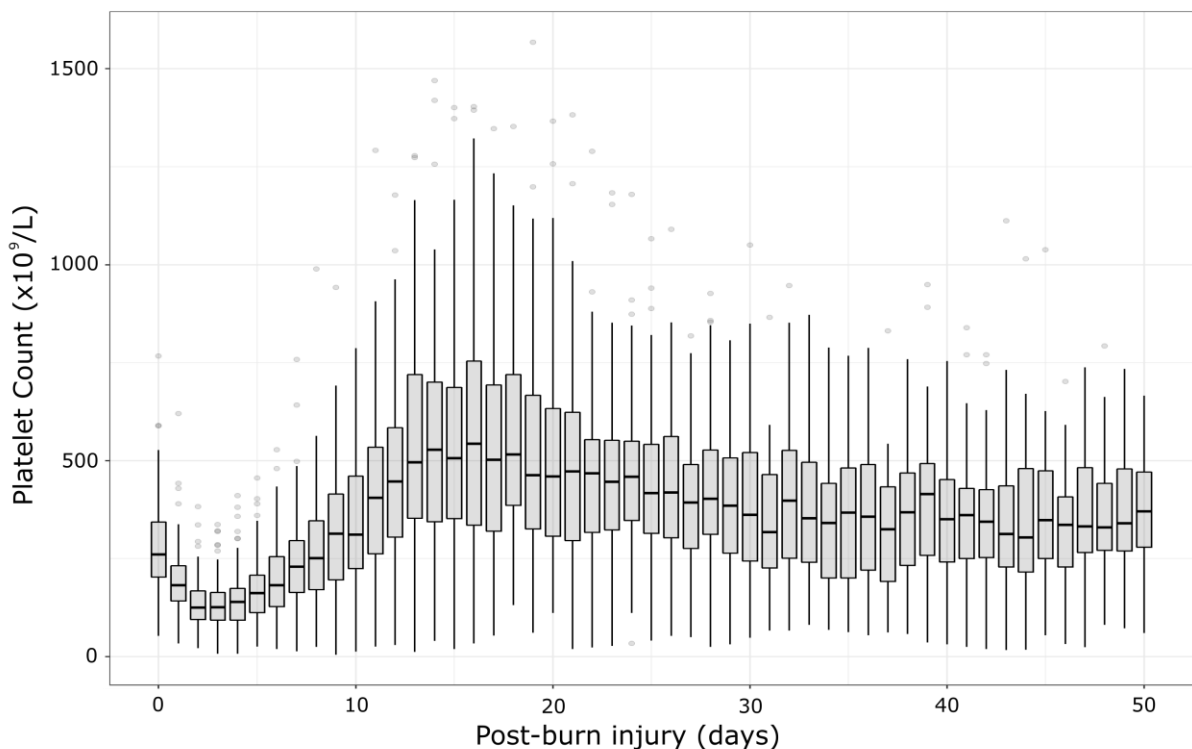
133 **Figure 1. Participant flowchart showing application of exclusion and inclusion criteria.**

134

135 Table 2. Demographics of study participants. BMI=body mass index; TBSA=total body surface area;
136 FT/DD=full thickness burn ABSI=abbreviated burn severity index; rBaux=revised baux score;
137 ICU=intensive care unit. *Missing data is due to death or discharge at the time of platelet peak count. ns
138 (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
139 where the median value is displayed and round brackets denote the SD where the mean value is
140 displayed.

141 ***Platelet count trajectories stratified by mortality or sepsis***

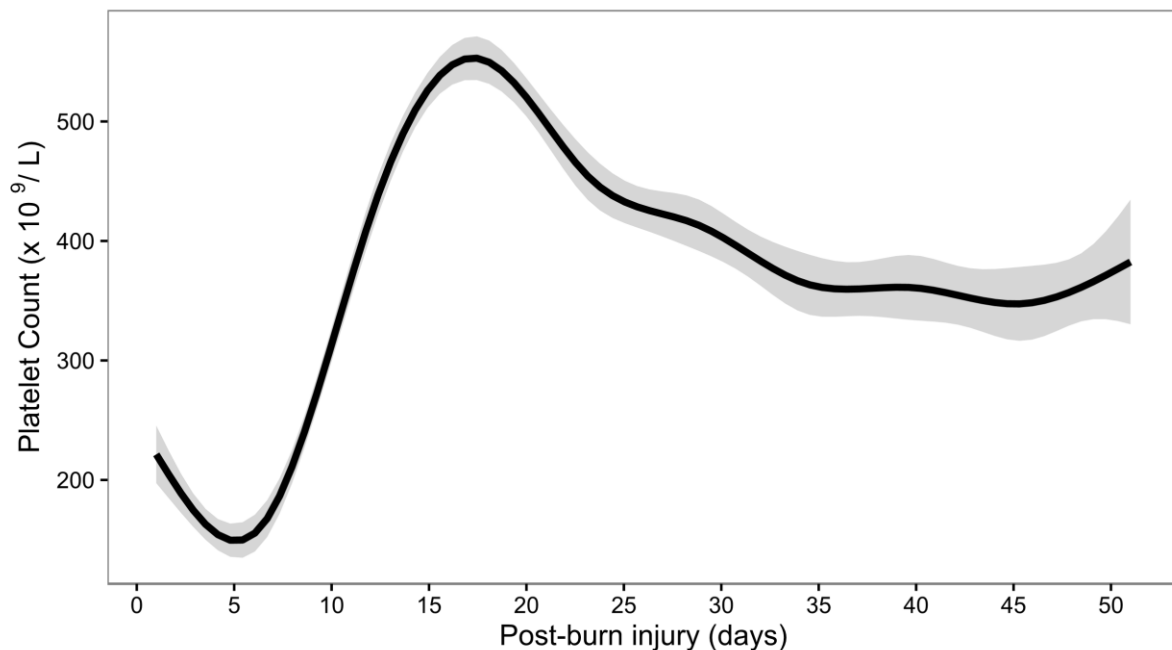
142 The time course of platelet counts are shown in Figure 2 and depict a classical
143 thrombocytopenic nadir which is between days 2-4 with a peak of thrombocytosis on days
144 11-17. Analysis of when each patient's individual platelet nadir occurs shows that, on
145 average, the nadir occurs on day 3. 57 (39.3%). Patients had their lowest platelet count on
146 day 2, and 41 (28.3%) had their lowest platelet count on day 3. Interestingly, high platelet
147 counts continue until day 50 post-injury without any indication of decline. This may be
148 artefact due to the decreased frequency of platelet count results at later time-points, where
149 the number of observations range between 114 and 128 across days 2 to 4 and between 77
150 and 99 across days 11 to 17. However, this difference is not discernible from Figure 2.



151

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

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

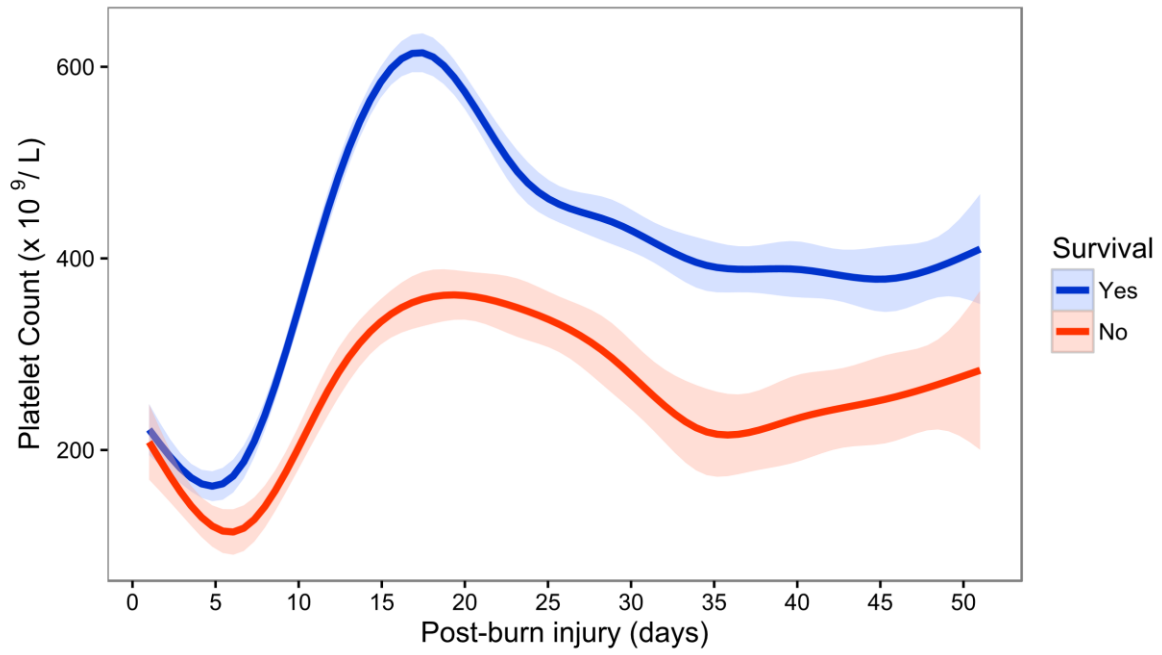


159

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

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

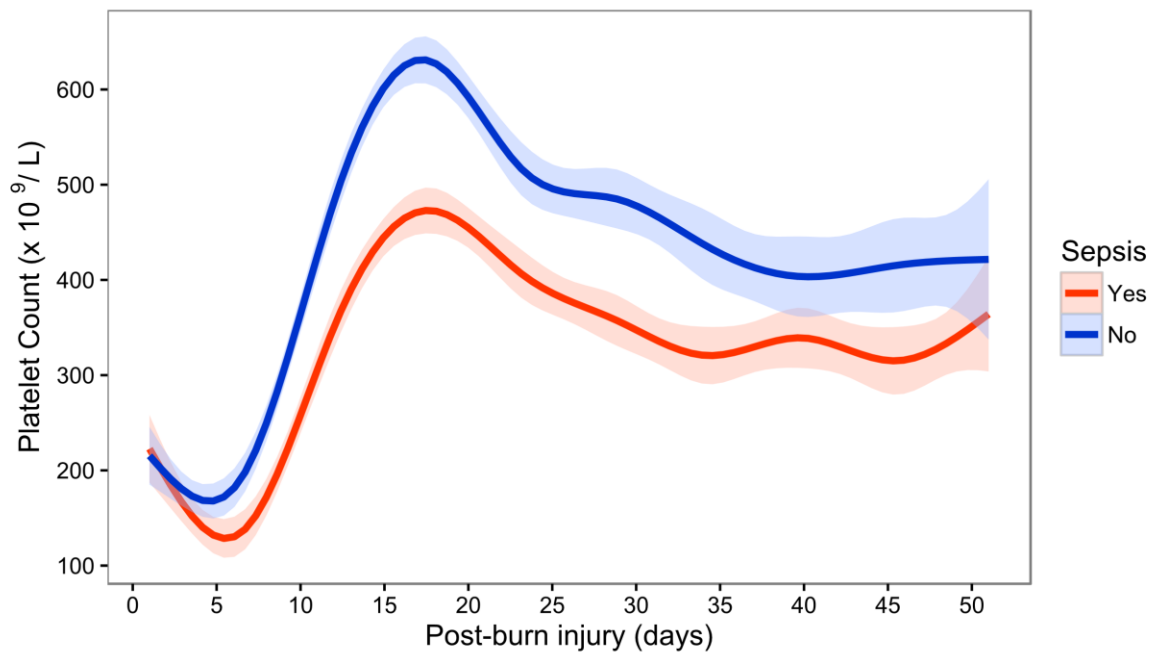
169 When stratifying the cohort by sepsis (Figure 5) the sepsis group reached lower platelet
170 count values at the nadir with marginal overlapping of confidence intervals. Similarly, to the
171 groups stratified by survival, patients with sepsis exhibit a significantly lower platelet count
172 peak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



173

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

176



177

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

180 **Daily models**

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

185 Table 3 shows the model outcomes for survival. Survival odds ratios are significant from day
186 2 post-burn in the nadir period and during all of the peak platelet count period even when
187 adjusted for rBaux score.

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

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

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

196 **Time to event analysis**

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

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

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

210 adjusted for rBaux the nadir platelet count still shows some relation to sepsis (HR=0.750
211 (95% CI 0.574-0.979).

212 **AUROC analysis**

213 Area under the receiver operator curve analysis revealed that the platelet count alone shows
214 poor discriminatory power for survival post-burn injury (Day 0, AUROC=0.534, 95%CI 0.387-
215 0.68). However, when combined with the rBaux score there is moderate discriminatory
216 power at less than 24 hours post-injury (AUROC=0.848, 95%CI 0.765-0.93).

217 In contrast platelet counts throughout the time course had limited power to discriminate
218 between septic and non-septic patients even when combined with the rBaux score (Day 0,
219 AUROC=0.742, 95%CI 0.648-0.835). Interestingly, the predictive power appears stronger
220 when combined with TBSA% rather than rBaux. On days 0, 3 and 14 the AUROC was 0.756
221 (95%CI 0.662-0.85), 0.779 (95% CI 0.697-0.862) and 0.776 (95% CI 0.676,0.876)
222 respectively showing poor to moderate discriminatory power for predicting sepsis.

223 **Other variables**

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

227 **Discussion**

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

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

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

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

253 There are various other factors that may affect platelet count. Drugs such as heparin can
254 cause a thrombocytopenia (Heparin Induced Thrombocytopenia (HIT)) but this is typically
255 later than we have seen in our cohort of patients, starting 5-10 days after the use of heparin
256 and hence is unlikely to contribute dramatically, if at all, to our observations[31].

257 Observations in published case reports have also suggested that in some patients
258 piperacillin-tazobactam, a commonly prescribed antibiotic, can cause thrombocytopenia but
259 these cases are very rare[32,33].

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

265 In our cohort there is an statistically significant difference in the platelet counts between
266 survivors and non-survivors in days 3-4 and indeed also in the peak platelet count, even
267 when corrected for the severity of injury using the rBaux score (OR=0.187 (95% CI 1.11-
268 3.15) and OR=0.175 (95% CI 1.10-2.80) respectively) (**Error! Reference source not
269 found.**). Indeed, this is also apparent from the Cox regression analysis (HR=0.813 (95% CI
270 0.756-0.874)). From previous burns literature, platelet count does appear to have some
271 relationship with mortality. Wang et al studied massive burns (>70% TBSA) in 102 adults,
272 and found that severe thrombocytopenia (platelet count < $20 \times 10^9/L$) was an independent
273 predictor of mortality ($p < 0.05$)[11]. However, this is quite a substantial thrombocytopenia
274 and such a substantial drop in platelet count is not frequently observed. Guo et al have also
275 demonstrated that a reduction in platelet count of greater than 65% from baseline is
276 predictive of 30-day mortality in burns patients ($p = 0.028$)[10]. It may be possible that the
277 bone marrow response to the initial platelet count drop is different in survivors and non-
278 survivors. This could be due to an enhanced inflammatory response in these individuals
279 stimulating bone marrow activity. Hence, measurement of both TPO and IL-6 levels over
280 time might also be very informative of the status of the megakaryocyte/platelet axis.

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

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

292 The platelet nadir also appears to have some association with sepsis. **The Cox regression**
293 **analysis shows significant values for platelet nadir with sepsis as the outcome (HR=0.750**
294 **(95% CI 0.574-0.979)), though data from the daily models adjusted with rBaux suggests this**
295 **effect is predominantly on day 3 (OR=0.58 (95% CI 0.39-0.85)). However, the AUROC data**
296 **suggests that this** is a poor to moderate predictor. The mechanisms behind this are largely
297 unknown however as discussed earlier, platelets have an important role in immunity. A lower
298 platelet count could lead to a compromised immune response to infection and increase
299 susceptibility to sepsis[36,37]. This is more likely than the converse, primarily due to the
300 early occurrence of the nadir; sepsis is more likely to develop later post-burn injury[38].

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

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

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

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

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

347 **Conclusions**

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

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

362

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368 commercial, or not-for-profit sectors.

369 **References**

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487

488

489 **Tables**

Included

Excluded

-
- | | |
|---|---|
| <ul style="list-style-type: none">• 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 | <ul style="list-style-type: none">• 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 |
|---|---|

490 Table 1. Inclusion and exclusion criteria.

491

	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 10⁹/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 10⁹ /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)	*

492

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

499

Day	Univariate Analysis			Adjusted for rBaux			
	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
	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
Peak	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

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

	Day	Univariate Analysis			Adjusted for rBaux		
		Sepsis OR	95% CI	p-value	Sepsis OR	95% CI	p-value
Nadir	2	0.83	(0.61, 1.13)	0.2428	0.86	(0.61, 1.19)	0.359
	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

502 Table 4. Daily odds ratios for sepsis from logistic regression analysis for days 2-4 (platelet
503 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

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

510 **Legends for Illustrations**

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

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

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

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

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