Histone-Associated Thrombocytopenia in Patients Who Are Critically Ill

Thrombocytopenia is observed in approximately 30% to 40% of patients in the intensive care unit (ICU) and associated with poor outcomes. Histones induce profound thrombocytopenia in mice and are associated with organ injury when released following extensive cell damage in patients who are critically ill. We explored the association between circulating histones and thrombocytopenia in patients in the ICU.

Methods | A case-control study was performed including patients admitted to the ICU at Royal Liverpool University Hospital between June 2013 and January 2014 with approval from the North West Centre of the Research Ethics Committees in the United Kingdom. Written informed consent was obtained.

Thrombocytopenia was defined as a platelet count less than 150 × 10^3/μL, a 25% or greater decrease in platelet count, or both within the first 96 hours of ICU admission (study duration). Patients with known prior cause(s) of thrombocytopenia were excluded. The control group was patients in the ICU without thrombocytopenia during the same study duration, matched to patients with thrombocytopenia for age, sex, APACHE II scores, and admission diagnoses. Plasma histones were measured, as described previously, and daily levels were compared between thrombocytopenic patients and nonthrombocytopenic control patients.

Table. Characteristics of Patients in the Intensive Care Unit

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients With Thrombocytopenia</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR), y</td>
<td>Mild (n = 18)</td>
<td>Moderate (n = 23)</td>
</tr>
<tr>
<td>Age, median (IQR), y</td>
<td>65 (48-71)</td>
<td>62 (49-76)</td>
</tr>
<tr>
<td>Men, No. (%)</td>
<td>13 (72)</td>
<td>14 (61)</td>
</tr>
<tr>
<td>APACHE II score, median (IQR)</td>
<td>17 (15-24)</td>
<td>21 (14-28)</td>
</tr>
<tr>
<td>Platelet count, median (IQR), × 10^3/μL</td>
<td>Admission</td>
<td>166 (139-224)</td>
</tr>
<tr>
<td>After admission</td>
<td>24 h</td>
<td>140 (130-152)</td>
</tr>
<tr>
<td>48 h</td>
<td>125 (117-136)</td>
<td>91 (73-107)</td>
</tr>
<tr>
<td>72 h</td>
<td>118 (107-129)</td>
<td>88 (77-100)</td>
</tr>
<tr>
<td>Plasma histone levels, median (IQR), μg/mL</td>
<td>Admission</td>
<td>4 (2-32)</td>
</tr>
<tr>
<td>After admission</td>
<td>24 h</td>
<td>5 (2-15)</td>
</tr>
<tr>
<td>48 h</td>
<td>15 (0-37)</td>
<td>22 (8-47)</td>
</tr>
<tr>
<td>72 h</td>
<td>22 (11-43)</td>
<td>17 (9-37)</td>
</tr>
<tr>
<td>Admission diagnosis, No. (%)</td>
<td>Sepsis</td>
<td>5 (8.9)</td>
</tr>
<tr>
<td>Trauma</td>
<td>3 (5.3)</td>
<td>5 (8.9)</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>4 (7.1)</td>
<td>4 (7.1)</td>
</tr>
<tr>
<td>Respiratory</td>
<td>2 (3.5)</td>
<td>3 (5.3)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>3 (5.3)</td>
<td>4 (7.1)</td>
</tr>
<tr>
<td>Renal</td>
<td>1 (1.8)</td>
<td>2 (3.5)</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>0</td>
<td>1 (1.8)</td>
</tr>
</tbody>
</table>

Abbreviation: IQR, interquartile range.

* Mild thrombocytopenia was defined as platelets 100 to 149 × 10^3/μL; moderate thrombocytopenia, platelets 50 to 99 × 10^3/μL; severe thrombocytopenia, platelets less than 50 × 10^3/μL.

* One P Value is provided if the P values for moderate vs mild and severe vs mild are the same.

* P values are presented for thrombocytopenia group (total) vs control group with no thrombocytopenia (total).

* Statistical significance determined by Mann-Whitney U test.

* Percentages are calculated as [number of patients in a particular diagnosis/56 (total number of patients) × 100].
controls. Because histones at approximately 30 μg/mL bind platelets and cause platelet aggregation, resulting in profound thrombocytopenia in mice,1,2 this level was used to stratify thrombocytopenic patients (high admission histones ≥30 μg/mL; low, <30 μg/mL). Platelet counts and percentage decrease in platelet counts at 24 hours and 48 hours after admission were compared between these 2 thrombocytopenic groups. In addition, daily histone levels were compared between patients with mild (platelets, 100-149 × 10³/μL), moderate (platelets, 50-99 × 10³/μL), and severe (platelets, <50 × 10³/μL) thrombocytopenia.

The Mann-Whitney U test was used for comparisons. The Receiver Operating Characteristic (ROC) curve assessed the performance of admission histone levels in predicting moderate to severe thrombocytopenia during the study. The χ² test was used for categorical groups (sex, presence or absence of a certain diagnosis, and presence or absence of circulating histones). Statistical tests were performed on SPSS software (IBM), version 22. A 2-sided P value less than .05 was considered significant.

**Results** | Fifty-six thrombocytopenic patients and 56 non-thrombocytopenic controls were studied. Circulating histones were detectable in 51 thrombocytopenic patients (91%) compared with 31 controls (55%) (P < .001). Daily histone levels were significantly higher in thrombocytopenic patients compared with controls throughout the study (Table).

Thrombocytopenic patients with high admission histones (n = 32) had significantly lower platelet counts at 24 hours and 48 hours after admission compared with thrombocytopenic patients with low admission histones (n = 24) (Figure, panel A). Thrombocytopenic patients with high admission histones had significantly greater percentage decreases in platelet counts at 24 hours and 48 hours after admission compared with thrombocytopenic patients with low admission histones (Figure, panel B).

Histone levels on admission and 24 hours after admission were significantly higher in patients developing severe or moderate thrombocytopenia compared with mild thrombocytopenia (Table). Admission histone levels were associated with development of moderate to severe thrombocytopenia with an area under the ROC curve of 0.893 (95% CI, 0.843-0.944, P < .001). At 30 μg/mL histone concentration, the sensitivity was 76% and specificity was 91% (predictive values: positive, 79.4%; negative, 89.2%; likelihood ratios: positive, 8.5; negative, 0.2).

**Discussion** | In this study, histones circulated in the majority of thrombocytopenic patients and were 2.5- to 5.5-fold higher than in nonthrombocytopenic controls. There was a significant association between high admission histones and subsequent decline in platelet counts among thrombocytopenic patients. High admission histone levels were associated with moderate to severe thrombocytopenia and development of clinically important thrombocytopenia with high area under the ROC curve.

The limitations of this study include a relatively small number of patients from a single center and the difficulty in establishing a causal relationship between circulating histones and thrombocytopenia without interventional studies.

Circulating histones are potential markers of disease severity,3,4,6 and the association with thrombocytopenia may reflect this. Nevertheless, the novel associations reported in this study extend previous reports demonstrating profound
thrombocytopenia following histone infusion into mice\(^1,2\) and suggest that, if confirmed, circulating histones may be valuable in predicting or monitoring thrombocytopenia in patients who are critically ill.

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Author Contributions: Drs Alhamdi and Toh had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Alhamdi, Wang, Toh. Acquisition, analysis, or interpretation of data: Alhamdi, Abrams, Lane, Wang, Toh. Drafting of the manuscript: Alhamdi, Wang, Toh. Critical revision of the manuscript for important intellectual content: Alhamdi, Abrams, Lane. Obtained funding: Wang, Toh. Administrative, technical, or material support: Wang. Study supervision: Wang, Toh.

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COMMENT & RESPONSE

School-Based Myopia Prevention Effort

To the Editor The school-based myopia prevention trial conducted by Dr He and colleagues\(^3\) supports what has been noted in observational studies\(^2\) that more time spent outdoors reduces the risk of myopia in children. However, one important issue regarding this finding has not been fully addressed by the authors.

Longitudinal cohort studies have indicated that more time spent outdoors has a significant protective effect on myopia onset, but not its progression.\(^3,4\) In other words, spending more time outdoors is only effective in children without myopia, but not in children with existing myopia.

In their analysis of 3-year changes in spherical equivalent refraction, which is an indicator for the progression of myopia, the effect size of the intervention was small and marginally significant (\(P = .04\)), consistent with current epidemiological data. Therefore, strategies for preventing myopia and retarding its progression should be different.

Further studies are warranted to investigate how to control myopia progression, which is important in reducing the burden of pathological myopia and other myopia-related ocular complications.\(^5\)

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To the Editor In their study on 6-year-old primary school children, Dr He and colleagues\(^4\) evaluated the effect of adding 40 extra minutes of outdoor activities to each school day on the 3-year cumulative incidence of myopia. The results showed that the cumulative incidence of myopia was 30.4% in the intervention group and 39.5% in the control group (\(P < .001\)).

Although the authors studied many potential confounders, they failed to document near-work activity, another important factor in the development of juvenile myopia.\(^2\) Several previous studies have established the role of near work on the development of myopia.\(^3,4\) Children who are within the ages evaluated in He et al’s study often are