Platelet abnormalities in experimental malaria—a review

EM Essien, OFR, FAS, NNOM (Nig), FWACP, MD.
(Lond).
Professor of Haematology, University of Uyo, Nigeria
University of Uyo Teaching Hospital, Uyo

Correspondence
emessien@yahoo.com
Department of Haematology
University of Uyo Teaching Hospital
P.O. Box 2407, Uyo, Akwa Ibom, Nigeria

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SUMMARY
Several abnormalities of platelet haemostatic function that have been previously described, are summarized in this article. These include hypersensitivity to agonists such as adenosine diphosphate (ADP), adrenaline and arachidonic acid; increased loss of platelet lactate dehydrogenase (LDH), and enhanced platelet production of thromboxane A2 (TXA2) found with enhanced but delayed prostacyclin (PGI2) production. Other features include marked reduction (by 36-62%) of total platelet sialic acid associated with shortened platelet life span. It was also observed that platelets that had interacted even for only one minute with Plasmodium falciparum-infected erythrocytes in culture, when challenged immediately afterwards with optimal concentration of ADP, had lost the usual platelet refractoriness after interaction with sub-optimal ADP concentration. The significance of this novel finding has not been investigated further.

These platelet changes which were also observed in the human disease, suggest that a hypercoagulable state associated with platelet activation exists in acute P. falciparum infection in man, a feature that was not previously described and appreciated. Its significance has not been investigated. However, it explains the relative rarity of bleeding complication that is sometimes described in the disease in man. Some of the changes described also suggest that additional mechanism(s) of platelet destruction in addition to those previously postulated, may occur in the infection. They also suggest that other therapeutic modalities may be available.

INTRODUCTION
A recent description of platelet abnormalities in acute malaria infection focused exclusively on data generated from human diseases. This resulted in inadequate appreciation of the range of platelet dysfunction in the infection and gave room for some of the inaccurate comments that were made. This preliminary review summarizes the platelet abnormalities observed in experimental malaria. It is hoped that it will avail all of the opportunities of these additional data and assistance to draw more balanced conclusions that would otherwise be difficult to arrive at using clinical data only.

THE INCIDENCE AND DURATION OF THROMBOCYTOPENIA IN ACUTE MALARIA
The few reports of changes in the platelet count in acute malaria infection in man invariably referred to thrombocytopenia and usually inferred that it occurred regularly in the disease.2,3 However, a more detailed analysis of the reports reveals that they usually described severe thrombocytopenia with platelet counts commonly below 30 x 10⁹ /L. It was suggested that this occurs in between 5% and 100% of patients.3 The wide range of incidence suggests, among other reasons, that there are problems such as the definition of ‘thrombocytopenia’ in view of the observation that normal platelet count is different for different populations,4 or other reasons.

In experimental studies utilizing Swiss albino mice or Syrian golden hamsters and P. bergei bergei (P. nigeriensis), it was shown that platelet count was regularly reduced in the infected animals. In Syrian
golden hamsters, platelet counts attained thrombocytopenic levels by the 6th post-infection day; by the 8th post-infection day, there was prolonged oozing from venepuncture sites; and by the 10th post-infection day, the animals were severely thrombocytopenic, with platelet counts of 175+/-.40, from pre-infection values of 622+/-83.5/10^9/L^3. With Swiss albino mice, the results were similar. As with hamsters, parasitaemia developed by the 2nd post-infection day, attaining significantly high levels (14-18%), by the 4th-6th post-infection day. Thrombocyte count correspondingly fell from a pre-infection level of 844.2+/-46.8 X 10^9/L to 500 X 10^9/L and to severe thrombocytopenic levels (317.5+/-62.8 X 10^9/L, P<0.005) by the 11th post-infection day. The thrombocytopenia persisted for as long as there was parasitaemia; but this usually cleared by days 14.-16, on the administration of intramuscular chloroquine for 7 days. (Our preliminary observations show that a 4 day course of chloroquine sulphate, as recommended, did not completely eliminate the parasitaemia in the peripheral blood). However, the thrombocyte count did not return to pre-infection values (844.2+/-46.8 vs 822+/-82.8 X 10^9/L until the 27th post-infection day. The delay may be a function of thrombocyte production in the animal species studied.

It should be noted that the animals usually died by the 6th or 7th post-infection day unless (as seen in the Swiss albino mice studies) they were treated.

These animal studies also clearly demonstrate the relationship between malaria parasitemia and thrombocyte count, and correlated with the few findings in human malaria infection that have addressed this specific question.

**DISORDERS OF PLATELET HAEMOSTATIC FUNCTION IN EXPERIMENTAL MALARIA**

The earliest report in this respect was the description of platelet hypersensitivity to exogenous adenosine diphosphate (ADP) or adrenaline in acute *P. falciparum* malaria infection in human malaria. A significantly enhanced aggregation response (hypersensitivity) of platelets as platelet-rich plasma (PRP), to exogenous adenosine diphosphate (ADP), of 39.8% or adrenaline when compared to the response in the control platelets of 5.2% (p<0.005) was demonstrated. This landmark finding has been regularly replicated by others but probably not all. Although the preparation of PRP is an elementary procedure, it is essential to pay close attention to such details as the standardization of platelet counts in control studies, and ensuring that the human subjects who donate their platelets had not taken anti-platelet drugs such as aspirin for at least a week before blood collection. In most developing countries where medical facilities are scarce, it is unlikely that a person who is ill with acute malaria would not have taken some drugs before arriving at the hospital where blood samples would be taken for tests, including tests of platelet function. Failure to pay attention to these critical details will ensure the inability to reproduce even the most elementary results that are almost routine in places familiar with platelet studies.

Platelet hypersensitivity to exogenous agonists has also been demonstrated in experimental animals and in an in vitro model. Other platelet function changes that have been reported in experimental malaria include enhanced production of thromboxane A2 (TXA2) and delayed but enhanced prostacyclin (PGI2). The delay in PGI2 production might be associated with the development of neointima, probably damaged early in the infection. In addition, the platelet lifespan in the animal model was shortened, and this was associated with total platelet sialic acid loss. Total platelet sialic acid loss of 8-10% had earlier been associated with rapid clearance of such platelets from the circulation.

It was shown in human malaria that elevated plasma concentrations of beta-thromboglobulin (BTG) and platelet factor-4 (PF4), as well as an increased loss of platelet lactate dehydrogenase (LDH) occurred in the infection. The elevated plasma levels of BTG and PF4 in platelet-rich plasma (PRP), as well as enhanced TXA2 production were taken to indicate that platelet hypersensitivity occurred in vivo in spite of the mean BTG: PF4 ratio of 1.8 (range up to 10).

**MECHANISMS OF PLATELET HAEMOSTATIC DYSFUNCTION IN EXPERIMENTAL MALARIA**

In the past, several mechanisms had been suggested to explain the thrombocytopenia which was, and still is, the focus of attention in malaria. These mechanisms include disseminated intravascular coagulation (DIC), immune-mediated mechanisms and active platelet ingestion of the parasites with resultant platelet destruction. The DIC mechanism is now accepted as being very rare. Platelet ingestion of a malaria parasite and its probable subsequent destruction was reported in just one study. Involvement of the immune mechanism in malaria-induced thrombocytopenia has been reported, but the mechanism(s) remains to be clarified.
Table 1. Summary of platelet changes in acute malaria infection

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Observation</th>
</tr>
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<tbody>
<tr>
<td>Platelet count</td>
<td>Deceased in all cases. Reduced to thrombocytopenic levels in 5-55% in <em>P. falciparum</em> (P.F) injection. Attains severe thrombocytopenia in 5-7% of cases in P.F.</td>
</tr>
<tr>
<td>Hypersensitivity</td>
<td>To ADP, Adrenaline (*P.F &amp; <em>p. nigeriensis</em>).</td>
</tr>
<tr>
<td>Plasma BTG &amp; PF4 concentration</td>
<td>Raised: 208.3+/-15.6 vs 59.2+/-15.7, p&lt;0.002</td>
</tr>
<tr>
<td>Plasma TXA2 concentration</td>
<td>Raised 113+/-83/plt/10^9 vs 30.05+/-21.8 pg</td>
</tr>
<tr>
<td>Pg12 concentration</td>
<td>Raised 170.3+/-32.7/plt/10^9 vs 148.5+/-23.1 pg</td>
</tr>
<tr>
<td>Platelet LDH loss</td>
<td>Increased loss</td>
</tr>
<tr>
<td>Platelet total Sialic Acid</td>
<td>Reduced by 36%-62% of normal</td>
</tr>
<tr>
<td>Platelet lifespan</td>
<td>Reduced</td>
</tr>
</tbody>
</table>

*Note:* BTG = beta thromboglobulin; *P. falciparum* = *Plasmodium falciparum*; TXA2 = thromboxane A2; PF4 = platelet factor; 3PGI2 = Prostacyclin

Platelet activation is a recent mechanism which has been suggested to account for the platelet findings in human malaria. Results of experimental studies have supported and have extended the research. Our pioneer studies, which have been replicated by others, have shown that platelet activation occurs regularly in acute malaria in man, in experimental animals and in in vitro models. The evidence has been summarized above. Some mechanisms of the activation such as the ADP mechanism (including loss of refractoriness induced by exposure to sub-optimal ADP concentration) and sialic acid loss have been investigated and proposed. However, many details of these mechanisms, especially at the molecular level, await investigation.

In addition, it is necessary to investigate the clinical applications of this knowledge so that possible new therapeutic strategies can be developed. This consideration becomes very important in view of the upsurge of malaria infection coupled with multi-drug resistance in a situation of very limited number of available drugs and irresponsible human behaviour in producing fake and sub-standard drugs. There is also the new suggestion that both malaria and HIV infections reinforce each other. In such a situation as this, we need to examine new directions of knowledge objectively.

A mild to moderate degree of thrombocytopenia (<90x10^9/L and <75x10^9/L) occurs in uncomplicated malaria. There is also the question whether the incidence and degree of thrombocytopenia are similar in infections with the different species of the human parasite as the reports have usually referred to *P. falciparum* infection (table 1).

Some of these questions have been addressed in more recent studies in different populations. For instance, the incidence of the thrombocytopenia in all grades of severity of acute malaria disease was 55% in *P. falciparum*, and 50% in *P. vivax*, infections. However the incidence of severe thrombocytopenia, (platelet count <30 x 10^9/L) was 5.5% 12 (table 1).

This wide range of the incidence is thought to be partly due to the fact that the normal platelet count varies in different populations.4

With respect to the involvement of immune-mediated mechanisms, the details of platelet biochemical changes need to be examined. For instance, platelet hypersensitivity response to exogenous agonists was demonstrated when washed normal human platelets were made to interact for only one minute, with washed *P. falciparum*-parasitized erythrocytes in culture (Essien, unpublished observations). It was shown that the ADP mechanism contributed 76-79% of the hypersensitivity response in vitro. These findings, contributed to the hypothesis that additional mechanism(s) involving ADP released from ruptured parasitized erythrocytes may play an important role in activating the platelets in vivo in acute malaria infection. Shortened platelet lifespan in acute malaria infection was also reported in man.

Although some recent studies have shown such findings do not necessarily suggest DIC in the absence of other evidence of the phenomenon, except in cases with severe thrombocytopenia which are usually associated with high blood parasitaemia (>20%). Elevated plasma fibrinopeptide A has been described in several clinical conditions, where it is usually an indicator of recent thrombin action on fibrinogen.

We are not aware that platelet response to agonists such as ADP has been tested in black water fever, a point seized on by critics of the hypothesis.

We did not determine total platelet sialic acid loss in this model.
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REFERENCES