

Platelet hyperactivity in sickle-cell disease: a consequence of hyposplenism

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SUMMARY Platelet function was measured on 29 occasions in 16 adult patients in the asymptomatic steady state of sickle-cell anaemia. There was a significant increase in platelet number and micro-aggregate formation, and a lower aggregation threshold with adenosine diphosphate, compared with 23 healthy controls. Similar changes were found, however, in 12 splenectomised patients without sickle-cell disease. The platelet hyperactivity of the sickle-cell steady state therefore reflects an increased circulating population of young, metabolically active platelets resulting from previous autosplenectomy.

Although the irreversibly sickled cell is the major cause of vascular obstruction in the painful vaso-occlusive crisis of sickle-cell disease, there is evidence that platelets are also involved. Liver biopsy during sickle-cell crisis has revealed platelet masses in association with aggregates of sickled cells,¹ the platelet count may fall during crisis compared with steady-state values,^{2,3} and there is a reduction in platelet survival.⁴ Clinical recovery is associated with a rebound thrombocytosis.^{3,5}

It is important to determine whether these changes are secondary to vascular stasis and endothelial damage during crisis⁶ or whether there is pre-existing platelet activation during the steady state which contributes to the onset of vaso-occlusive crisis. Published reports of platelet function in asymptomatic sickle-cell disease have given conflicting results: platelet aggregability has been reported as increased,⁷ normal,^{4,8} and decreased,⁹ with either no change⁴ or decreased aggregability^{8,9} during crisis. There is, however, one report of aspirin-dipyridamole prophylaxis causing a small reduction in symptomatic episodes in three patients.¹⁰

The present study was designed to examine platelet function during the steady state in comparison with healthy controls and splenectomised patients.

Patients and methods

Blood samples were obtained, without venostasis,

from 16 patients with sickle-cell disease (12 S/S, three S/ β thalassaemia, and one S/C disease) of mean age 20.7 (range 15-42) years at routine outpatient visits. Each patient was studied on an average of two occasions in the steady state and had been asymptomatic for at least four weeks before testing; it has been shown that the haemostatic abnormalities, including rebound thrombocytosis, of sickle-cell crisis return to normal within four weeks.⁵ None of the patients had palpable splenomegaly. Blood was obtained under identical conditions from a control group of 23 healthy subjects of mean age 24.7 (range 19-30) years. The tests which showed a statistically significant difference between these two groups (see Table) were subsequently performed on blood from 12 splenectomised patients without sickle-cell disease of mean age 19.9 (range 7-33) years. The splenectomy had previously been performed, as part of the staging procedure for lymphoma, at least three months previously, and the patients were not currently receiving cytotoxic drugs or radiotherapy. All the patients had also avoided, for at least two weeks, any drugs that interfere with platelet function.

Platelet counts, using EDTA-anticoagulated blood, were performed using the Thrombofuge-Thrombocounter system (Coulter Electronics Ltd). Blood for platelet function studies was collected into 1/10th volume of 3.13% trisodium citrate in polystyrene tubes and centrifuged once at 22°C and 200 g for 10 minutes, to produce platelet rich plasma (PRP), and then for 15-20 minutes at 22°C and 1500 g, to obtain platelet-poor plasma (PPP). The platelet counts of PRP were adjusted to $300 \times 10^9/l$

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Table Mean (\pm SD) results for the sickle-cell steady state compared with healthy controls and postsplenectomy patients

	Healthy controls		Sickle-cell steady state		Postsplenectomy
Platelet count ($\times 10^9/l$)	(23)* 232 ± 68		(29) 388 ± 141		(12) 465 ± 197
P		< 0.001		NS	
In-vivo microaggregate ratio	(21) 0.88 ± 0.13		(23) 0.68 ± 0.22		(12) 0.62 ± 0.23
P		< 0.001		NS	
ADP-threshold (mmol/l)	(23) 1.77 ± 0.80		(29) 1.36 ± 0.71		(12) 1.37 ± 1.18
P		< 0.05		NS	
Spontaneous aggregation (%)	(23) 6.2 ± 4.8		(29) 6.6 ± 4.7		
P		NS			
Heparin thrombin clotting time (s)	(23) 30.1 ± 12.8		(29) 36.6 ± 20.3		
P		NS			

*Number of tests in parentheses.

using autologous PPP, kept in capped polystyrene tubes at room temperature, and tested within 45 minutes.

Spontaneous platelet aggregation was performed by a modification of the method of Wu and Hoak¹¹ using 0.5 ml PRP mixed for 10 minutes at 700 rpm and at 37°C in a dual-channel platelet aggregometer (Malin Electronics Ltd, Ayr, Scotland) attached to a Vitatron 2001 recorder (MSE-Fisons Ltd, Crawley, Sussex). Transmission was adjusted to 0% for PRP and to 100% for PPP. The change in transmission was recorded as percentage spontaneous aggregation.

The minimum adenosine diphosphate (ADP) concentration required to produce a biphasic aggregation response (ADP threshold) was determined by adding 0.1 ml ADP (BDH Chemicals Ltd, Poole, Dorset) to 0.5 ml PRP to give final concentrations ranging from 0.2 to 5.5 mmol/l PRP.

Platelet *in vivo* microaggregate ratios were measured by the formalin-fixation method of Wu and Hoak¹² but using twice the volume of blood and buffer to improve accuracy.

The heparin thrombin clotting time (platelet factor 4-like activity) was determined according to O'Brien *et al.*¹³ using a thrombin concentration of 6 units/ml and adjusting the heparin concentration to give a control time of 25 seconds for pooled normal plasma.

Statistical significance was determined by the Mann-Whitney U test.

Results

Patients with sickle-cell disease, compared with

healthy controls, showed a significantly higher platelet count, lower *in vivo* microaggregate ratio, and lower ADP threshold (see Table). There was no significant difference in spontaneous aggregation or heparin thrombin clotting time.

When the results for sickle-cell patients were compared with those for postsplenectomy patients, there was no significant difference in platelet count, *in vivo* microaggregate ratio, or ADP threshold.

Discussion

This study of asymptomatic sickle-cell anaemia has shown a significant increase in platelet number and microaggregate formation and a lower threshold for ADP-induced aggregation, compared with healthy controls of similar age. The raised platelet count has previously been reported¹⁴⁻¹⁷ and an increase in large platelets (megathrombocytes) was also demonstrated in one study.¹⁶ It is particularly important to select appropriate control patients in studies of sickle-cell disease,¹⁸ and when we included a second control group of adult asplenic patients we could no longer demonstrate these differences in platelet number and function.

Functional asplenia, as determined by reduced uptake of isotope, develops in childhood sickle-cell disease¹⁹ and is followed by ischaemia-induced fibrotic atrophy.²⁰ Splenectomised adults, without sickle-cell disease, have a thrombocytosis^{21, 22} caused by loss of the normal splenic platelet pool which contains up to one-third of the total body platelet mass.²³ Absence of this pool has also been demonstrated in sickle-cell anaemia.²⁴ The spleen has been

shown to retain newly formed platelets for up to two days before their release into the circulation²⁵ and to contain many large platelets²⁶ which are young,²⁷ more active metabolically,^{28 29} and show greater responsiveness in platelet function tests.^{30 31} The few studies of platelet function after splenectomy have shown normal adhesiveness^{32 33} but increased aggregation with ADP and also collagen.³³

Thus the apparent platelet hyperactivity of the sickle steady state reflects the absence of splenic pooling of young active platelets rather than chronic intravascular activation of platelets in the micro-circulation. Although a recent study of 318 post-splenectomy patients showed no significant association between postsplenectomy thrombocytosis and thromboembolism,³⁴ the increased number of circulating active platelets in sickle-cell disease may readily lead to aggregation in areas of stasis during vaso-occlusive crisis. This is consistent with the fall in platelet count,^{2 3} reduction in platelet survival,⁴ and decrease in aggregability of the remaining circulating platelets.^{8 9}

Platelet hyperactivity in the steady state, and also platelet activation during crisis, now appear to be secondary phenomena, and it seems unlikely that antiplatelet therapy could have a major effect in reducing the frequency or severity of sickle-cell crises.

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