Are anticardiolipin antibodies responsible for some of the complications of severe acute *Plasmodium falciparum* malaria?

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Abstract **What were first called simply false-positive Wassermann reactions and then lupus anticoagulant are now known as antiphospholipid or anticardiolipin antibodies (ACA). These are known to cause a tendency to thrombosis and are frequently present in many neurological conditions and infections. The pathological significance of these antibodies in acute infections, if any, is unknown. We investigated the presence of these antibodies in *Plasmodium falciparum* malaria in an endemic area in Natal/KwaZulu, and attempted to correlate the presence of this antibody with cerebral manifestations. Immunoglobulin G-anticardiolipin antibodies measured by enzyme-linked immunosorbent assay occurred significantly more frequently in 62 patients with acute *Plasmodium falciparum* malaria (33,9%) than in 37 control subjects (2,7%) ($P < 0,0001$). There was no significant difference in the mean parasite loads in those patients who were positive for ACA (1,75%) and those who were negative (1,59%) ($P = 0,83$). No correlation was found between parasite load and ACA levels in the patient group, or between the number of cerebral manifestations in patients with and without the antibody. The frequency of splenomegaly was not significantly different in patients with and without ACA ($P = 0,06$). We conclude that there is a high prevalence of ACA in acute falciparum malaria. The pathological significance of this antibody and its relationship to complications, especially cerebral ones, warrant greater attention and may improve the understanding of cerebral malaria and its management.**

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Anticardiolipin antibodies (ACA) occur frequently in a wide variety of infections,[1] including AIDS,[2] mycoplasma infections,[3] malaria[4] and Lyme disease.[5] The pathological significance of the anticardiolipin antibody in acute infections, if any, is unknown. The possible role of these antibodies in neurological diseases has been especially emphasised.[6] These antibodies have been detected in focal cerebral ischaemia, the myelopathy of lupoid sclerosis and Dego's disease, Guillain-Barré syndrome, migraine, chorea, and seizures.

Approximately 110 million cases of malaria occur each year, 90 million in tropical Africa. Global deaths are estimated at approximately 1 million a year,[7] usually from *Plasmodium falciparum* infection. The mortality rate for cerebral malaria ranges from 10% to 50% in treated patients.[8] Obstructed microcirculatory flow due to 'sludging' of red cells in capillaries and venules is thought to be responsible for many of the severe manifestations of acute falciparum malaria.[9] Nevertheless, most survivors have no easily detectable neurological deficit.[10] Better understanding of the pathology of cerebral malaria could lead to more effective therapy and an improved survival rate.

In this paper, we report on the high prevalence of ACA in patients with acute falciparum malaria and attempt to correlate the presence of the antibody with manifestations of cerebral involvement.

Patients and methods

Sixty-two consecutive patients with confirmed *P. falciparum* malaria attending a clinic in an endemic area in Natal/KwaZulu were studied. Thirty-seven healthy volunteers from the same area were also studied for comparative purposes. Blood smears from this control group were all negative for parasites of the plasmodium species.

At presentation, a detailed history was taken from each patient and a physical examination performed. Particular note was made of any cerebral symptoms, such as confusion, drowsiness and convulsions. The parasite load was quantitatively analysed on thin smears of blood.

Serum samples from all patients and controls were tested for the presence of immunoglobulin (IgG)-ACA by means of a routine enzyme-linked immunosorbent assay (ELISA). Results were expressed in IgG phospholipid (GPL) units by relating the optical density readings to those produced by a reference serum provided by the Rayne Institute, London.[11] Values of less than 10 GPL units were within the normal range. The Wassermann reaction was tested for on all sera; *Treponema pallidum* haemagglutination (TPHA) and rapid plasma reagin (RPR) tests were done on all positives. In the presence of a positive definitive serological test for syphilis, an accompanying positive ACA test was regarded as a false-positive.

The χ^2-test was used for comparison of categoric variables and Student's *t*-test for comparison of continuous variables. Elevated ACA levels were correlated with the parasite load, the presence of cerebral symptoms and the presence of splenomegaly.

Results

Of the 62 patients assessed, 28 were men and 34 women. The mean age was 21 years (range 5 - 60 years). In the control group, 10 were men and 27 women. Their mean age was 26 years (range 6 - 60 years). The mean parasite load in the patient group was 1,62% (range 0,1 - 10,74%).

Twenty-three patients were positive for ACA (Table I). Two of these sera tested positive for syphilis, giving a true-positive rate of 21/62 (33,9%). Of the 37 controls, 3 tested positive for ACA, but 2 of them had positive Wassermann reaction and RPR results. The incidence of ACA in controls was thus 2,7% (1/37). This difference in ACA positivity between patients and controls was highly significant ($P < 0,0001$). The odds ratio for ACA positivity was 18,44 (95% confidence interval: 2,361; 144,012). The ACA levels for the patients and controls expressed in GPL units are shown in Fig. 1. Values greater than 10 GPL units are elevated.

TABLE I.
Anticardiolipin antibodies in 62 falciparum malaria patients and 37 controls

	Patients	Controls
ACA-positive	23	3
False-positive*	2	2
True-positive	21 (33,9%)	1 (2,7%)

* Positive serological tests for syphilis.

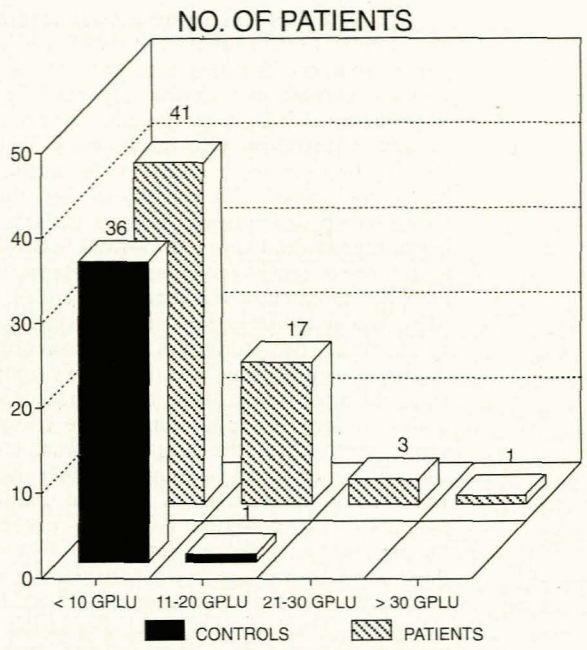

FIG. 1.
Anticardiolipin antibodies levels in 62 patients with *P. falciparum* malaria and 37 controls. Values higher than 10 GPL units are considered elevated.

There was no statistically significant difference in the mean parasite load between the 21 patients who were positive for ACA (1,75%; SD 3,26) and the 42 who were negative (1,59%; SD 2,13) ($P = 0,83$). No correlation was found between parasite load and ACA levels in the patient group (Pearson's $r = 0,029$). Table II shows the number of cerebral manifestations in patients with and without ACA. There was no significant difference between these two groups. Splenomegaly was found in 14/21 patients with ACA (66,7%) and 17/41 patients without ACA (41,5%) ($P = 0,06$).

TABLE II.
Number of cerebral manifestations in 21 patients with and 41 without ACA

Cerebral manifestations	ACA-positive	ACA-negative
0	10	23
1	3	9
2	6	9
3	2	0

Discussion

Severe and complicated malaria has recently been defined and theories of cause debated.[9] Included are complications such as cerebral malaria, renal failure, pulmonary oedema, disseminated intravascular coagulation (DIC) and ischaemic necrosis of the liver. All these are thought to be caused by problems in the microcirculation. At present several theories have been put forward to explain these lesions, e.g. the sludging hypothesis, the permeability hypothesis and various mechanical hypotheses. Two popular theories include cyto-adherence of infected red cells and the effects of cytokines, such as tumour necrosis factor (TNF), which are known to cause adherence of leucocytes and erythrocytes. In our paper we put forward the hypothesis that anticardiolipin antibodies may be one of the factors responsible for microcirculatory thrombosis in severe and complicated malaria.

The results of this study show that there is a high prevalence (33,9%) of ACA in patients with acute falciparum malaria. A prevalence of 2,7% in our control group is consistent with that reported for the general population.[12] The significance of this antibody in malaria is unknown. Our results show no definite relationship between the presence of the antibody and cerebral complications. To our knowledge, the only other study which determined ACA in malaria sera[4] found levels higher than those in normal controls but lower than those in sera of patients with systemic lupus erythematosus; no attempt was made to correlate these results with clinical manifestations of the infection.

However, this study was a cross-sectional one conducted at a rural clinic and none of the patients studied were followed up to determine whether more severe manifestations, such as unarousable coma, developed subsequently. Also, the sample was small and no significant difference could be found in either the mean parasite load or the number of cerebral manifestations in patients with and without ACA. It is uncertain whether the antibody is, wholly or partly, directly responsible for the complications seen in this infection, or whether they are merely an epiphenomenon secondary to polyclonal activation. The lack of a direct correlation between the parasite load and ACA level suggests that this phenomenon may not be related to severity of infection alone.

The association between the ACA and thrombocytopenia is a well-recognised phenomenon.[13] Thrombocytopenia is also common in falciparum malaria[14] and is not a particular feature of severe infection.[15] It has been suggested that ACA may bind phospholipids in platelet membranes resulting in enhanced uptake and destruction by the reticulo-endothelial system.[16] Skudowitz *et al.*[17] have shown that thrombocytopenia in falciparum malaria is the result of excessive splenic pooling as well as decreased platelet survival. Unfortunately, platelet counts could not be measured in our study.

In patients with systemic lupus erythematosus a close association exists between ACA and the so-called lupus anticoagulant which, in spite of its *in vitro* effects, acts as a risk factor for vascular thromboses. The presence of widespread microthrombi and obstructed microcirculatory flow in affected organs in falciparum malaria[18] suggests that the ACA may play some role in the pathogenesis of the complications. This may involve exacerbation of the thrombotic tendency caused by knob proteins which appear on infected red cells and are thought to be responsible for cyto-adherence to endothelial cells.[19] It is also possible that certain moieties of the ACA may react with epitopes on cerebral phospholipids, such as sphingomyelin, and thereby contribute to neurological complications.

Further studies of larger patient groups are required to compare the prevalence and levels of ACA in complicated and uncomplicated malaria. This would be useful in assessing whether ACA has any prognostic significance in patients who present early with acute falciparum malaria. In addition, it would be valuable to determine whether changing levels could be used to monitor progress in these patients.

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