Measurement of acute phase proteins for assessing severity of *Plasmodium falciparum* malaria

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Abstract

Seventeen adult patients with acute *Plasmodium falciparum* malaria, admitted to the Hospital for Tropical Diseases, were studied. Serial measurements of the serum concentration of C-reactive protein, serum amyloid A protein, and percentage parasitaemia were determined, together with initial measurement of serum electrolytes, liver function, haemoglobin, white cell and platelet counts. Initial C-reactive protein and serum amyloid A concentrations were increased (C-reactive protein mean 49.0 mg/l serum amyloid A 28 mg/l) falling towards the normal range by the seventh day of treatment. There was a significant correlation between the pre-treatment parasite count and clinical and laboratory markers of inflammation. C-reactive protein and serum amyloid A concentrations correlated inversely with the serum sodium.

These results indicate that measurement of acute phase reactants such as C-reactive protein and serum amyloid A may prove valuable in assessing the severity of *P falciparum* malaria, and in following the response to antimalarial treatment.

The pathophysiological response to infection with *P falciparum* is highly variable. It depends on several different factors including previously acquired immunity and the production of cytokines such as interleukin-1 (IL-1) and tumour necrosis factor (TNF), γ -interferon (γ -IFN), and interleukin-6 (IL-6). Secretion of these mediators is thought to be responsible for many of the clinical features of malaria.¹⁻⁶

Clinical assessment of severity depends on bedside observation of variables such as blood pressure, fever, and mental alertness. These can be supplemented by routine laboratory measurements. The degree of parasitaemia is correlated with severity of disease and falls in serum sodium and albumin are often found in more severely ill patients.⁷

C-reactive protein is a pentameric protein molecule which has been conserved over a long period of evolution. It binds to phosphorylcholine epitopes found in *Streptococcus pneumoniae* and many parasites. Binding results in activation of the classical complement pathway. Serum amyloid A is the

precursor of amyloid A protein found in the amyloid fibrils present in secondary amyloid deposits. Both proteins increase in concentration in the serum up to 1000 times in response to inflammatory stimuli, whether infective or traumatic.⁸

Measurement of acute phase reactants like C-reactive protein or serum amyloid A has been used to monitor the response to antibacterial treatment. Increased concentrations of C-reactive protein have also been used to distinguish acute pyogenic from viral meningitis.⁹ Although the stimulus to acute phase reactants production is not fully understood, macrophage derived cytokines including IL-6, TNF, and IL-1 are thought to be important.¹⁰

Measurement of cytokines has been used in studies of malaria in children in the Gambia and Malawi^{2,11} and adults in Germany.⁴ Measurement of cytokine concentration is not usually available in clinical laboratories. The very short half-life of cytokines in serum makes assay of these substances difficult to interpret in clinical practice. This study was designed to test whether measurement of acute phase reactants as an indirect measure of cytokine concentration would prove valuable in assessing the severity of malaria compared with standard laboratory markers, and whether sequential measurement would be valuable in assessing response to antimalarial treatment.

Methods

Seventeen patients with uncomplicated acute *P falciparum* malaria, admitted to the Hospital for Tropical Diseases, London, were studied. Twelve were men and five women. Twelve were of European origin, four were African, and one was from the Indian subcontinent. Sixteen of the patients acquired their infection in Africa and one in the Far East. Malaria was the only diagnosis in each case and all were successfully treated with intravenous or oral quinine followed by three tablets of Fansidar.

The following laboratory investigations were performed on the day of admission: percentage parasite count, differential white cell count, measurement of the haematological indices including platelet count, together with measurement of serum electrolytes, bilirubin, and liver enzyme concentrations. The patient's maximum temperature was recorded. Blood was drawn daily and a percentage parasite count performed. C-reactive protein and serum amyloid A were measured on daily

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Figure Serum concentrations of C-reactive protein and serum amyloid A in 17 patients with acute *P falciparum* malaria. Mean value indicated by solid line.

serum samples using a competitive enzyme linked immunosorbent assay (ELISA) technique.

Immulon 2 (Dynatech) microtitre plates were coated with affinity purified antibodies to human C-reactive protein and serum amyloid A (derived in rabbit and goat, respectively) by incubation overnight at 4°C in bicarbonate buffer (pH 9.2). Serum specimens were diluted in phosphate buffered saline with 0.05% Tween-20 (pH 7.5) (PBS-Tween). Diluted sample (100 µl) was added to a microtitre well together with 100 µl of alkaline-phosphatase conjugated C-reactive protein or serum amyloid A and incubated for one hour at room temperature in a humidified chamber. The optimal concentration for all of the reagents was previously determined by chequerboard titration. After washing the plates p-nitrophenyl phosphate substrate was added, and the resulting colour change measured using a Dynatech 600 ELISA reader at 405 nm. The concentration of C-reactive protein or serum amyloid A present in the sample was determined by calculation from a series of standard concentrations of highly purified C-reactive protein or high density lipoprotein (HDL) tested in parallel on the same plate.

Results

Fifteen of the 17 patients had raised concentrations of C-reactive protein (>10 mg/l) and

serum amyloid A (>4 mg/l) on presentation. The mean concentration of both acute phase reactants increased on the second day of treatment, then fell slowly over the next five days. Individual values show variation but with a general downward trend in parallel with the disappearance of parasites from the peripheral blood and clinical recovery. The results for all patients are shown in the figure.

No patients had gross abnormality of biochemical and haematological indices, although several had increased bilirubin concentrations and decreased serum sodium. This was consistent with the clinically mild course of the infection in this patient group. The mean of all results, together with range and standard error of mean, are recorded in table 1.

Correlation was sought between serum concentration of acute phase reactants C-reactive protein and serum amyloid A and the conventional marker of malaria severity—parasite count, and with haematological and biochemical variables. There was no correlation between the peripheral white cell count and any other variable measured. A significant positive correlation was found between percentage parasite count and acute phase protein concentrations. There was a highly significant inverse correlation between serum sodium, the maximum temperature, the parasite count and the concentration of acute phase reactants. A significant positive correlation was also found between the maximum temperature and the concentration of C-reactive protein and serum amyloid A, and with serum bilirubin concentration. These results are summarised in table 2.

Table 1 Mean values of parasitological, haematological, and biochemical variables measured in 17 patients presenting with acute *P falciparum* malaria

Investigation	Mean	Range	SEM
C-reactive protein	46.1	(3.4–242 mg/l)	14.8
Serum amyloid A	28.8	(1.4–158 mg/l)	8.7
Sodium	136	(126–141 mmol/l)	0.9
Bilirubin	22.5	(8–60 mmol/l)	3.24
Albumin	39.8	(35–47)	1.02
White cell count	5.5	(3.3–7.8)	0.3
Platelets	159.6	(59–327)	19.74
Maximum temperature	38.3	(36–40.2)	0.28
Percentage parasitaemia	1	<0.1–4.8%	0.33

Discussion

The methods used to assess objectively the severity of *P falciparum* malaria on admission, and the response to antimalarial chemotherapy are unsatisfactory.¹² Severity can be assessed by evaluating a combination of clinical observations including height of fever, disturbance of

Table 2 Summary of correlation coefficients between laboratory markers of severity in 17 patients with acute *P falciparum* malaria

	Sodium	Maximum temperature	White cell count	Parasitaemia	Albumin	Bilirubin	Platelets	C-reactive protein
Serum amyloid A	-0.735***	0.545*	0.142	0.713***	-0.105	0.448	-0.178	0.949***
C-reactive protein	-0.802***	0.588*	0.104	0.676**	-0.134	0.336	-0.238	—
Platelets	-0.077	0.163	0.182	0.014	-0.012	-0.019	—	—
Bilirubin	-0.124	0.539*	0.340	0.226	0.204	—	—	—
Albumin	0.456	-0.292	-0.243	-0.334	—	—	—	—
Parasitaemia	-0.831***	0.617**	0.105	—	—	—	—	—
White cell count	-0.008	-0.013	—	—	—	—	—	—
Maximum temperature	-0.667***	—	—	—	—	—	—	—
Sodium	—	—	—	—	—	—	—	—

***p < 0.001

**p < 0.01

*p < 0.05

conscious level, hypotension, or detecting other signs indicating the development of complications. Laboratory markers have also been studied, and severity of malaria has been associated with higher parasitaemia, a fall in serum sodium and albumin concentration, low platelet counts and a rise in serum bilirubin and urea concentrations. Measurements of parasitaemia have drawbacks as markers of severity as semi-immune people may tolerate levels of parasitaemia which would be associated with severe disease in non-immune subjects. The study reported here used non-immune subjects with mild to moderate infection, in whom malaria was the only diagnosis, to reduce non-specific confounding effects.

Recent research has confirmed the importance of leucocyte mediators such as TNF in the pathogenesis of severe malaria. Even in severe disease cytokine concentrations remain in the picogramme per ml range. This low concentration is coupled with a short plasma half-life, making measurement difficult in the clinical setting. A study of TNF concentrations in 75 Gambian children with acute *P falciparum* malaria failed to show a correlation between the concentration of TNF and severity of disease, although increased TNF concentrations were found more often in patients with complications.² In a study in Germany of patients with *P falciparum* and *P vivax*, however, serum IL-6, measured using a bioassay and TNF measured by ELISA, was shown to be correlated with the presence of severe complications. Moreover, serum concentrations fell in response to antimalarial chemotherapy.⁴ In a study of 65 children with severe *P falciparum* malaria in Malawi the mean initial serum concentration of TNF was significantly higher in the 10 patients who died than in the 55 who survived.¹² High concentrations of TNF were also associated with hypoglycaemia, hyperparasitaemia, and severity of illness as measured by a prognostic index.

A secondary consequence of cytokine production is the stimulation of the acute phase reaction. Studies of sequential acute phase reactant concentrations have been correlated with severity of bacterial infection and therapeutic response to specific antibacterial treatment.

This pilot study shows significant correlations between conventional laboratory markers of severity of malaria—parasite count, serum sodium and albumin—with acute phase reac-

tants. This may be of value in supporting the clinical assessment of patients and in more accurately classifying patients being entered into clinical trials.

Current methods for assessing the response to antimalarial chemotherapy are imprecise.¹² Several different variables have been used. Parasite clearance time is widely accepted, but it is comprised by the sensitivity of the test and observer error.¹³ Studies vary in their definition of parasite clearance time, some choosing the time to the last positive slide and others the time to the first negative slide. Observer errors are particularly important in studies of mild malaria when parasite numbers are low. Other measures which have been used in the assessment of chemotherapy include mortality as an end point. It has been shown, however, that the use of death as an end point would require very large patient numbers to show a significant difference between regimens.¹² Other clinical measures of therapeutic response include time to abatement of fever, but accurate consistent measurement of fever is difficult in large studies. Complex manipulations of fever data are compromised by the initial difficulty of obtaining consistent data. It is therefore difficult to compare fever data among different studies.

It has been suggested that laboratory variables which show improvement in response to treatment, such as urea, glucose, platelet count, etc, would be valuable in the assessment of response to antimalarial chemotherapy but this possibility has not been shown in prospective studies.

Each of the patients in this study showed a consistent fall in acute phase reactant concentrations over the period of antimalarial treatment. Although individual values could vary, sometimes increasing, there was a consistent downward trend. The concentrations of acute phase reactants were found to be increased on the second day of the hospital admission in all patients studied; this is consistent with an increase in parasitaemia which occurs commonly up to 18 hours after starting antimalarial chemotherapy.

These data support the idea that sequential measurement of acute phase reactant concentrations are valuable in the assessment of the clinical course of patients with acute *P falciparum* malaria. Clinicians monitoring treatment in this way must be alert to the variation which occurs and observe the overall trend.

Such measurements have physiological relevance as they are an indirect measure of inflammatory mediators thought to be important in the pathogenesis of the disease. Consequently, measurement of acute phase reactant concentrations are likely to be more objective than clinical observation in a clinical trial setting. We would expect, therefore, that the use of sequential measurement of acute phase reactants as a means of assessing response to chemotherapy will result in more reproducible results among different centres and among trials.

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