Red Cells Disorders

Leukocyte alkaline phosphatase in *Plasmodium falciparum* malaria

We studied leukocyte alkaline phosphatase in malaria to assess leukocyte defence mechanisms. Twenty–seven patients with malaria were stratified into two classes on the basis of disease severity. Fifteen malaria negative patients were taken as controls. Data showed mild polymorphonucleated cell activation, in the absence of correlation with the severity of the malaria.

The metabolic burst of polymorphonucleated cells (PMN) is significantly increased in patients with acute malaria, and it is therefore possible that PMN have a role in defence against malaria.[1] Plasma concentrations of PMN elastase, myeloperoxidase and lysozyme, and urinary concentrations of neopterin were found to be elevated in patients with severe *Plasmodium falciparum* malaria and to be related to the severity of the disease.[1-4]

The enzyme leukocyte alkaline phosphatase (LAP) is located in neutrophils.[5] LAP increases following PMN activation, e.g. during infections[6] or after stimulation by granulocyte colony-stimulating factor.[7] LAP activity may therefore be interpreted as a sign of PMN activation. No data concerning LAP in malaria infection are available.

A study was carried out in Mugana hospital in north Tanzania in 1997. We enrolled 27 inpatients with malaria (26 children aged 2 months to 6 years and one adult). Clinical examination was performed to exclude concomitant infectious diseases. Another 15 inpatients, aged 3 to 11 years, in whom malaria was excluded, were taken as controls (Table 1). The same physician controlled thick and thin blood smears to make the diagnosis of malaria, and patients were stratified into two classes on the basis of the percentage of trophozoites, and the WHO clinical criteria for severe *P. falciparum* malaria (Table 1). The group of malaria negative patients (group 0) was subdivided into a group of malaria negative/non-infectious patients (group 0a), hospitalized for minor surgery, minor traumatic disease, malnutrition, and a group of malaria negative/infectious patients (group 0b), predominantly with acute bronchitis, measles, and infectious diarrhea. Hemoglobin concentration, LAP score, differential leukocytic count, and a count of peripheral neutrophils containing visible pigment were determined in all patients.

Blood smears were stained with May-Grünwald–Giemsa and LAP stains, according to the Kaplow protocol.[8] As indicated by the protocol, 100 neutrophilic leukocytes for each smear were scored from 0 to 4+ on the basis of the intensity of dye precipitation in the cytoplasm. All smears were read by the same physician, as recommended. The total score is the sum of the individual 100 cell scores. A normal LAP value (Kaplow index) is between 60 to 90.

The percentage of trophozoites was determined in May-Grünwald-Giemsa stained smears by counting all *P. falciparum* plasmodia in 10 homogeneous fields at 1000 X magnification. The percentage of neutrophils containing visible pigment was determined on the same smears.

The results are presented in Table 1. A correlation between mean LAP value and body temperature was observed only for malaria negative/infectious patients, whereas normal or slightly increased LAP values were observed in patients admitted for other diseases. No correlation between LAP values and severity of malaria was observed. A significant increase in PMN percentage was observed only in the group of septic patients; whereas in the overall study population relative neutropenia was more frequent. When the patients were stratified on the basis of age instead of the WHO severity criteria, still no correlation was noted between LAP score and age.

PMN activation has been postulated to occur in malaria infection on the basis of plasma concentrations of myeloperoxidase, lysozyme,[3] and elastase .[2] As eosinophil and monocyte/macrophage populations, all of which show myeloperoxidase activity, are activated during malaria infection, specific activation of PMN is difficult to infer on the basis of the plasma enzyme levels alone. High myeloperoxidase levels in association with elevated lysozyme and neopterin levels could be ascribed to monocyte/macrophage activation.[4] Plasma elastase levels could also be ascribed to cells other than PMN.

LAP values, however, do appear to be a valid indicator of PMN activation, and could, therefore, be useful for determining the true degree of PMN activation, as the quantification is made directly within the cells.

Our data clearly show that even though mild PMN activation occurs during malaria, there is no correlation between PMN activation and the severity of the malaria. Cellular activation thus appears aspecific and could be attributed to one or more cytokines normally present during the malaria infection. Absence of major PMN activation in malaria could be ascribed to parasite phagocytic suppression mechanisms or, alternatively, to the fact that PMN are only marginally involved in the control of malaria, while monocytes are much more critical in this disease. Perhaps significant recruitment of PMN only occurs in very severe forms of malaria. LAP values could be influenced by body

Table 1. Characteristics of patients and controls.

	Age	Sex (M/F)	Temperature (°C)	Parassitemia %	PMN %	Hb g/dL	Impaired consciousness	LAP score
Group 0	6.6±2.47	9/6	(a) 37.1±0.9 (b) 38.7±0.52	(a) 0 (b) 0	(a) 56 (b) 83	13.2±1.5	0 %	(a) 78±16 (b) 327±41
Group 1	5.2±3.1	5/4	37.2±0.78	0.8±0.25	57	12.5±1.8	0 %	166±18
Group 2	3.2±2	11/7	38.8±1.04	2.7±0.47	46	6.6±2.1	22 %	176±z5

Parassitemia: mean percentage of red blood cells containing Plasmodium falciparum trophozoites; all results are expressed as mean ± standard deviation. Group 0 is constituted of a group of malaria negative/non-infectious patients (sub-group a) and a group of malaria negative/infectious patients (sub-group b).

temperature, but within the group of patients with malaria no correlation was observed with body temperature.

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References

1. Gilpin-Dzhekson E, Shadrin BP. The nitroblue tetrazolium reduction test in evaluating the function of the circulating leukocytes in mice with experimental malaria. Biull Eksp Biol Med 1988; 105:166-8.

2. Pukrittayakamee S, Clemens R, Pramoolsinsap C, Karges HE, Vanijanonta S, Bunnag D, et al. Polymorphonuclear leukocyte elastase in Plasmodium falciparum malaria. Trans R Soc Trop Med Hyg 1992; 86:598-601.

3. Mohamed AO, Elbashir MI, Ibrahim G, Ismail M, Venge P. Neutrophil leukocyte activation in severe malaria. Trans R Soc Trop Med Hyg 1996; 90:277.

4. Brown AE, Webster HK, Teja Isavadharm P, Keeratithakul D. Macrophage activation in falciparum malaria as measured by neopterin and interferon-γ. Clin Exp Immunol 1990;82:97-101.

5. Valentine WN, Beck WS. Biochemical studies on leukocytes. I. Phosphatase activity in health, leukocytosis, and myelocytic leukemia. J Lab Clin Med 1951;38:39-44.

6. Valentine WN, Folette JH, Solomon DH, Reynolds J. The relationship of leukocyte alkaline phosphatase to "stress", to ACTH, and to adrenal 17-OH-corticosteroids. J Lab Clin Med 1957;49:723-9.

7. Rambaldi A, Masuhara K, Borleri GM. Flow cytometry of leukocyte alkaline phosphatase in normal and pathologic leukocytes. Br J Haematol 1997;96:815-22.

8. Kaplow LS. A histochemical procedure for localizing and evaluating leukocyte alkaline phosphatase activity in smears of blood and marrow. Blood 1955;10:1023.

Acute Myeloid Leukemia

Molecular monitoring to identify a threshold of CBFβ/MYH11 transcript below which continuous complete remission of acute myeloid leukemia inv(16) is likely

Many patients with the inv(16) positive acute myeloid leukemia (AML) achieve complete remission (CR). Using real-time reverse transcriptase polymerase chain reaction (RT-PCR), we previously proposed critical CBFβ-MYH11 transcript copy number thresholds to predict relapse or cure. We now update the molecular follow-up of our patients, also presenting the therapeutic management of these patients.

We extend the molecular follow-up of 17 patients with inv(16) acute myeloid leukemia (AML) for whom cytogenetic and molecular follow-up data are available, dividing the group into those who relapsed and those who have not. Having monitored the expression of CBFβ-MYH11 during the follow-up we establish a value below which continuous complete remission is likely.

Although the outcome of AML is predominantly related to age,[1] it is of prime importance to distinguish subgroups of patients with different risks of disease. Cytogenetic analysis at diagnosis is generally considered the single most valuable prognostic factor in AML;[1,3] in particular, patients with t(8;21), t(15;17), or inv(16) were found to have a relatively favorable prognosis.[2,3] In the case of acute promyelocytic leukemia, molecular monitoring of minimal residual disease (MRD) status by qualitative or, preferably, quantitative real time PCR (RQ-PCR) analysis of the PML-RAR transcript can provide a suitable tool for predicting relapse and offering indications for allogeneic transplantation.[3] In the case of inv(16) positive AML the prognostic significance of the different levels of CBFβ-MYH11 transcript copy number in monitoring MRD remains to be defined.[4]

To provide further information on the threshold of CBFβ-MYH11 transcript copy number above which relapse occurs and below which continuous complete remission (CR) is likely, in the present work we update the follow-up analysis of our series of patients[5,6] with inv(16)-positive AML patients treated with different therapies (one cycle of induction therapy and one or two cycles of consolidation therapy, autologous bone marrow transplantation in 13 patients and allogeneic bone marrow transplantation in 3 patients). The clinical data are summarized in Table 1.

All 17 patients underwent molecular and cytogenetic analysis during the follow-up. Samples of bone marrow (n=201) and peripheral blood (n=6) were collected for routine care of the patients. All RQ-PCR experiments were performed at least in triplicate. Since January 2000, we also applied the same RQ-PCR conditions and protocol for the CBFβ/MYH11 fusion transcript as those currently recommended by the *European Concerted Action (EAC) of standardization of fusion transcripts in AML patients* (i.e. CBFβ-MYH11 per 10^4 copies of transcript copy numbers normalized to the ABL housekeeping gene). Comparisons of the CBFβ-MYH11/ABL ratios at diagnosis, relapse and during treatment and CR were performed by the Kruskal-Wallis test. The χ^2 test was used for binary variables. All analyses were performed using the SPSS software package (SPSS Inc., Chicago, IL, USA).

Fifteen patients are currently alive (88%) either in first CR (n=12, 70.5%) or second CR (n=3, 17.6%). The response rate after induction therapy was 100%. Four patients of the seventeen (29%) had a relapse but three (11%) went on to achieve a second CR lasting >36 months. The median overall survival (OS) was 62 months (range 37-140) with median disease free survival (DFS) in 1st or 2nd CR being 56 months (range 36-109).

We retrospectively analyzed the CBFβ-MYH11/ABL ratios of peri-/post-treatment samples from 17 patients using RQ-PCR. The 17 patients were divided into non-relapsing and relapsing subgroups. All ratios <12 (minimum, 0) obtained during or after treatment (n=101) belonged to patients who have continued to remain in long-term CR without relapsing. By contrast, all ratios >25 (maximum, 710) obtained during or after treatment belonged to patients who went on