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Peripheral parasite density and its relationship to severity of disease in pediatric cerebral malaria

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ABSTRACT

PERIPHERAL PARASITE DENSITY AND ITS RELATIONSHIP TO SEVERITY OF DISEASE IN PEDIATRIC CEREBRAL MALARIA. Rachel N. Bronzan. Kilifi Coastal Unit, Kenya Medical Research Institute, Kilifi, Kenya. (Sponsored by Frank J. Bia, Department of Medicine, Yale University School of Medicine, New Haven, CT).

Pediatric cerebral malaria is an important cause of pediatric mortality in sub-Saharan Africa. Many efforts have been made to determine risk factors for serious disease and to elucidate prognostic indicators, but studies to date have generally shown little or no correlation between peripheral parasite density and severity of disease or outcome. This study attempts to further define the relationship between parasite density and disease, specifically investigating the interactions of age, parasite density, and mortality in cases of cerebral malaria versus noncerebral malaria patients. Our data set is larger than any which has been previously analyzed, and includes peripheral parasite counts on 3281 children ages 0 to 13 years, including 2212 inpatients (419 cerebral malaria, 1257 severe noncerebral malaria, and 479 anemic patients), 438 outpatients, and 631 healthy children from the community. For each group of patients, the geometric mean parasite density at each age was determined and parasite densities were compared among groups. Mortality was analyzed as a function of both age and parasite density for each group, and the parasite densities for those who died were compared with the counts of those who survived for each group. Parasite density was slightly positively correlated with age for all groups analyzed together ($r=0.09$, $p<0.0001$) and for noncerebral patients, but not for other groups. Cerebral malaria and anemic patients' parasite densities tended to decrease with age. Although inpatients with severe disease had significantly higher geometric mean parasite densities as compared to other inpatients, cerebral and anemic patients had significantly lower parasitemias than noncerebral patients with severe disease. Since mortality is highest in the former two groups (cerebral patients 15.75% and anemic

patients 6.1%), peripheral parasite density alone at time of admission is clearly not of prognostic value for death. Additionally, because of the wide range of parasite densities within each group, parasite density is also not predictive of severe disease. Thus, this study, more powerful than previous ones of its type, corroborates previous findings that higher peripheral parasite density is grossly associated with worse prognosis but that peripheral parasite density is of no prognostic value except at extremely high levels. Modifiers of the parasite density/disease relationship and other determinants of morbidity and mortality are discussed.

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INTRODUCTION

Malaria is a leading cause of morbidity and mortality in the developing world. It has been estimated that there are 300 hundred million people infected with malaria in the world and 100 million cases of malaria in developing countries each year [1]. In children alone there are between 0.5 and 2 million deaths a year in sub-Saharan Africa [2]. In malaria-endemic areas severe disease, characterized mostly by severe anemia and cerebral malaria, is seen primarily in children and accounts for approximately 1-2% of all malarial disease in children [2]. Cerebral malaria carries case fatality rates between 6 and 50% in the pediatric population, with many of those who survive having permanent neurological sequelae [3, 20, 21, 22, 27, 28].

The pathogenesis and pathophysiology of cerebral malaria are not fully understood. Sequestration of parasitized red blood cells (PRBCs) in the cerebral vasculature of patients is strongly implicated in the pathogenesis of cerebral malaria, but the pathophysiology of clinical disease is poorly understood. It has been shown, however, that patients with cerebral malaria sequester more red blood cells (RBCs) than do patients with other forms of malaria [4] and that the degree of PRBC sequestration is strongly correlated with a clinical coma scale [5]. Whether the mass of sequestered infected RBCs is directly a function of the total body parasite load, or whether host-parasite interactions (i.e. specific interactions between infected red blood cells and cerebral post-capillary endothelium) primarily determine the degree of sequestration, has yet to be determined. If total body parasite load is correlated with degree of sequestration, one might expect a strong positive association between high peripheral parasitemias and cerebral malaria. If specific cell-cell interactions are a strong determinant in the degree of sequestration, one could postulate at least a weakening of such an association between parasitemia and disease. Certainly a combination of factors contribute to sequestration and disease.

Many efforts have been made to determine risk factors for serious disease and to elucidate prognostic indicators. There have been varying reports on whether parasite density correlates with severity of disease in patients without cerebral malaria, and there are even fewer data concerning this potential correlation in patients with cerebral malaria [3,14,18,38,11,16,35]. The goal of this study was to determine if there is a correlation between high peripheral parasite counts and cerebral malaria in a malaria-endemic area. We studied children in coastal Kenya who were infected with *Plasmodium falciparum* and specifically examined the interrelationships between parasite density, type and severity of clinical disease, age, and mortality.

Life Cycle of the Malaria Parasite

In order to understand the issues surrounding this study, it is necessary to understand the life cycle of the malaria parasite in man. *Plasmodium falciparum* is the most virulent of the four species of human malaria and is the sole focus of this study. Infection with *P. falciparum* begins with the bite of an infected anophelene mosquito, in our instance *Anopheles gambiae*. In the process of obtaining a blood meal, the female mosquito deposits sporozoites into the host. The median size of this "dose" per bite is approximately 20 sporozoites [6]. The sporozoites then pass to the liver where they invade hepatocytes and multiply 10,000-fold over a period of about 8 to 9 days. The parasites, now called merozoites, rupture from the hepatocytes and are released into the bloodstream to invade erythrocytes of all ages. This begins the erythrocytic stage of infection, the stage associated with disease [7].

Once inside the red blood cell the parasite develops through the stages of ring form and trophozoite, and finally reproduces to form about 20 merozoites packed into a schizont. The schizont eventually ruptures, releasing the merozoites into the bloodstream, increasing the parasite population within the host. Less commonly the parasite differentiates into gametocytes, the sexual form of the parasite. The parasite's

life cycle is completed when a female mosquito takes another blood meal and ingests the gametocytes, which are infectious for mosquitoes.

The entire cycle within the red blood cell transpires in 48 hours. During the second half of the process, erythrocytes containing trophozoites adhere to the endothelium of the post-capillary venules in the deep vascular beds of organs throughout the body, a process known as sequestration. Consequently, only the younger ring forms of the *P. falciparum* parasite are seen on examination of peripheral blood [7].

Clinical Presentation of Malaria

The clinical presentation of malaria is varied. Symptoms of uncomplicated malaria include fever, malaise, nausea, vomiting, and headache. However, in malaria-endemic regions there is a high prevalence of asymptomatic parasitemia, so diagnosis of malarial disease is not straightforward. The presenting symptoms are shared with many other common diseases, and the prevalence of parasitemia in people with fevers can be expected to be at least that which is found in the general population [8].

Severe Disease: Presentation and Potential Risk Factors

In children, severe disease has two distinctive presentations, severe anemia and cerebral malaria [8]. Severe disease occurs in a minority of children, but what determines whether or not severe disease will develop is still poorly understood [2,8]. Hypoglycemia, convulsions, septicemia, retinal hemorrhages, elevated CSF lactate, and hyperparasitemia (≥20-25% cells parasitized [9,10]) are all associated with poor prognosis [10]. In Malawi, Molyneux *et al* used a retrospective analysis to develop a bedside prognostic index which identified those children with cerebral malaria who were at increased risk for adverse outcome (death or neurologic sequelae) [3]. This index included parasitemia $>1 \times 10^6$ ring forms/μl, blood glucose ≤2.2 mmol/l, and white blood cell count $> 15 \times 10^9$/l. Age has also been repeatedly shown to be a risk factor not only for malarial disease but for *severe* malarial disease as well. Younger

children have more clinical malaria episodes per year than do older ones. Interestingly, although children under the age of one year are at highest risk for severe anemia, it is those over the age of two who are at highest risk for cerebral malaria [7, 8]. This unusual association of age with the spectrum of disease will be considered in more detail later.

Severe anemia is attributable not only to the destruction of parasitized red blood cells by the spleen but also to the destruction of nonparasitized red blood cells, as well as to depressed erythropoeisis [23] The degree of anemia has often been referred to as being disproportional to the level of parasitemia [24]. However, a recent paper modeling the interaction between parasites and erythrocytes using coupled differential equations, suggests that severe anemia can result from low parasitemia even with constant erythropoeisis [23]. Anemia is often severe enough to require transfusion, and it is not uncommon for anemia to trigger heart failure.

The clinical features of cerebral malaria include a range of neurological findings, and the diagnosis will vary with the clinical criteria being used [25]. In the research setting coma scales are used to produce more uniform diagnoses, but rigorous inclusion criteria may exclude true cases [8] while loose clinical definitions may lead to elevated estimates of its incidence. Cerebral malaria is often characterized by sudden onset. Clinical features include coma, seizures, and other neurologic findings such as gaze abnormalities, retinal hemorrhages, and decorticate or decerebrate rigidity. Postmortem pathologic examination of the brain often confirms the diagnosis of cerebral malaria.

Although a positive blood smear helps to make the clinical diagnosis, it is possible to have a transiently negative blood smear in a semi-immune person if an infection is highly synchronized and the parasitized cells are all sequestered. Other causes of coma and seizure confuse the picture. The Glasgow coma scale is poorly suited to the assessment of coma in pediatric cerebral malaria patients. The Blantyre

coma scale [3], developed in Blantyre, Malawi, is shown below and is far more appropriate for assessing young children in coma because it does not require that children be able to speak or follow commands. Additionally, children in malarial coma often have consistently open eyes even in the absence of corneal reflexes, indicating that this is not "spontaneous eye opening", yet these children would score favorably on the Glasgow coma scale.

Blantyre Coma Scale

Best motor response	Score
Localizes to painful stimulus	2
Withdraws limb from painful stimulus	1
No response or inappropriate response	0
Best verbal response	
Cries appropriately with painful stimulus or, if verbal, speaks	2
Moan or abnormal cry with painful stimulus	1
No vocal response to painful stimulus	0
Eye movement	
Watches or follows (e.g. mother's face)	1
Fails to watch or follow	0

A score of 0 in any of the three components of this coma scale is predictive of poor outcome, but a combined score of 0 correlates more strongly with outcome [3].

Laboratory Diagnosis

Laboratory diagnosis of malaria typically involves the use of thick and thin blood films. Although other diagnostic methods are available, such as polymerase chain reaction, blood films are the most practical method available for use in the field. Thick films employ larger samples of blood than do thin films and are therefore useful in rapid screening for parasites. Preparation involves spreading out the blood on a slide, allowing it to dry, and then lysing the red blood cells before staining. This allows quantification of parasite density (parasites/μL blood) by calculating the

number of parasites observed per 100 white blood cells and then multiplying by the patient's white blood cell count.

For higher levels of parasitemia a thin blood film is the preferred method for quantitative diagnosis. These films use a very thin layer of blood, and permit the direct visualization of parasites within red blood cells. This allows the estimation of the percent parasitemia (the percent of the total red blood cells which are infected) and again permits calculation of the parasite density by counting the number of parasites observed per 500 red blood cells and then multiplying by the patient's red blood cell count.

Common laboratory findings in malaria include anemia, acid/base disturbances, hypoglycemia, elevated CSF lactate, and elevated white blood cell count. The latter three findings are associated with poor outcome. Acid/base disturbances, hypoglycemia, and elevated CSF lactate in particular are common findings in cerebral malaria.

Pathogenesis and Pathophysiology of Cerebral Malaria

As mentioned previously, the pathogenesis and pathophysiology of cerebral malaria are poorly understood. The pathophysiology is probably related to sequestration, cytoadherence of PRBCs to the cerebral vasculature, but the mechanisms responsible for the pathology are not fully defined. Risk factors for development of cerebral malaria may be contributed by both the host and the parasite [2, 7]. For the host these include HLA type, lack of immunity to certain strains of malaria parasites, and sensitization by previous exposure [2, 7, 29]. For the parasite these include exofactors (soluble parasite factors which may be toxins or may induce cytokine production), ability to induce tumor necrosis factor (TNF), and capacity to bind to endothelium and form rosettes [7, 26, 34]. These factors will be elaborated upon further in the discussion.

Sequestration is a process peculiar to *Plasmodium falciparum* in which infected RBCs adhere to the endothelium of deep vascular beds and reduce blood flow. As described, sequestration occurs about 24 hours into the 48 hour life cycle of the malaria parasite. Mature trophozoites and schizonts are found sequestered in the deep vascular beds of many different organs, while only ring stage parasites are found in the peripheral blood. Sequestration can occur in any organ, but when it occurs in the brain, cerebral malaria can result.

The process of sequestration clearly has some potential benefits for the parasite [8]. First, parasitized cells evade the spleen by residing in deep vascular beds. Second, by obstructing blood flow sequestration produces a more hypoxic and acidic environment which is beneficial to the asexual growth of the parasite. Third, it has been postulated that sequestration provides for more efficient infection of new RBCs, because newly released merozoites encounter a higher proportion of uninfected RBCs when they enter the peripheral circulation [8].

On electron microscopic examination, parasitized red bloods cells exhibit small protrusions on their cell membranes which are termed knobs. These knobs are neoantigens, and have been shown to be parasite antigens which have been transported to the cell membrane by the intracellular parasites [30], and they may also be modified host RBC determinants [39]. In cerebral malaria it is these antigens which adhere to various receptors on the endothelial cells of the cerebral vasculature, primarily the post capillary venules, as well as to nonparasitized red cells (termed rosetting) [7], both of which probably contribute to the gross process of sequestration [31, 36].

Some neoantigens are highly conserved while others demonstrate the ability to rapidly switch phenotypes, even in the absence of selective pressure [32]. The ability to rapidly change these surface antigens could clearly be of importance in the parasite's efforts to evade the host's immune response and this has implications for the development of vaccines directed at intracellular stage parasites. Furthermore, it has

been demonstrated that the adhesive potential of these antigens changes with the phenotype [32] which suggests a role for these changes in the pathogenesis of disease, a role separate from immune evasion.

On the cerebral endothelium there are several candidate receptors for parasitized RBC neoantigens including thrombospondin, CD36, ICAM-1, E-selectin, and vascular cell adhesion molecule-1 [7, 8]. The interaction of the parasite surface antigen polymorphisms described above with host receptor polymorphisms could result in extreme person-to-person variation in the severity and type of disease [2]. These interactions will also be elaborated upon in the discussion.

Peripheral Parasite Density and Disease

What is the relationship between peripheral blood parasite density and disease severity? Parasite prevalence in a population and average parasite density in individuals are indices which are often used to demonstrate malaria endemicity [8]. But the relationship of these factors to clinical disease has not been clearly demonstrated, so their usefulness as surrogate measures of morbidity in the analysis of the effects of various interventions is questionable.

It has been demonstrated in The Gambia that while clinical disease from malaria shows striking seasonal variation, parasite prevalence varies little over the year, suggesting that parasite prevalence does not correlate well with the amount of disease in their population [15]. This illustrates the difficulties in distinguishing between malarial infection and malarial disease. In Kenya a mean of approximately 33% of well children had parasites on a thick smear [9]. In such a setting, a positive malaria blood film in a patient with fever may be an incidental finding as the prevalence of parasitemia in patients with fever will always be at least as high as the prevalence of parasitemia in the general population. These kinds of data have led some researchers to try to define a numerical cut-off level of parasitemia above which asymptomatic

infection is uncommon [33, 15]. Exactly where this cut-off should fall is difficult to determine. And as mentioned, severe disease does occur in patients with low, or even transiently absent, parasitemias while patients with higher parasitemias can be asymptomatic [14].

Age of the infected individual also influences the relationship between parasitemia and disease. In malaria endemic regions, there is clearly a decrease in the frequency and severity of disease at older ages where higher parasitemias are tolerated without disease, suggesting that partial immunity may be established over time. Although parasitemia occurs at all ages, the majority of symptomatic disease is seen in children. Mortality from malaria is highest in children under the age of five, before the age at which peak parasite prevalence is reached. Greenwood [15] and Molyneux [3] have both found that in ill children parasite density does not correlate with age. In the latter, study age <3 years was associated with poor outcome, demonstrating an effect of age on disease which is independent of parasitemia.

Finally, only a few studies have specifically examined the relationship between parasite density and severity of disease. Conclusions have been conflicting as to the prognostic value of parasitemia at time of admission in predicting severity of disease or outcome. In 1937, Field and Niven found a close correlation between mortality and parasitemia, but only for those cases in which treatment was not given prior to doing the blood smear. They also noted a >50% mortality in those patients with more than 500,000 parasites/μL [38]. These findings led to the acceptance and use of peripheral parasitemia at time of admission as a gross indicator of morbidity and mortality. But there have been many cases in the literature illustrating that very high parasitemias can be tolerated without significant disease, and that severe disease or even death can occur in the presence of very low parasite counts [14]. Field further supported the correlation between parasitemia and mortality in 1949, and another study may corroborate these findings [18]. A recent publication suggests that geometric mean

parasite density in the general pediatric population can be used as a surrogate measure of morbidity and mortality from malaria and would thus be a useful measure when assessing various interventions, but this may only apply to mild forms of disease. Molyneux et al [3] found an increased risk for adverse outcome such as death or neurologic sequelae for those pediatric cerebral malaria patients with parasitemias over $1x10^6/\mu L$.

In 1949 Field noted that peripheral schizogony is associated with higher parasitemias, yet felt that peripheral parasite density was of more prognostic value than was peripheral schizogony. A mathematical model has been proposed and tested which correlates higher proportions of late stage parasites in the peripheral blood with degree of sequestration and thus severity of disease [12, 13].

Several studies found no correlation between admission parasitemia and severity or type of disease [11, 16], although one study found that parasitemias in asymptomatic people were significantly lower than those parasitemias associated with disease [11]. Another study suggests that disease severity correlates better with the sequestered parasite load than the number of circulating parasites [35]. Some of these studies were limited by the number of patients (insufficient power) or the methods used in collecting or analyzing data.

Our study attempts to further define the relationship between parasite density and disease, looking specifically at the interactions of age, parasite density, and mortality in cases of cerebral malaria versus noncerebral malaria and malarial anemia. We examined our data to see if there was a difference in the mean parasite density in cerebral malaria patients as compared to severely ill but noncerebral malaria patients and additionally looked at mortality to see if it was associated with a higher parasitemia when compared with children of the same age and disease status. Few if any prior studies have had sufficient patient numbers to look at the association between parasitemia and mortality.

METHODS

Study Area, Population and Hospital

All patients in this study were from Kilifi District on the coast of Kenya. The majority of the population are Giriama, members of the Mijikenda ethnic group. A 1989 national census estimated the total population of the district to be 63,834 persons, 79% of whom are rural farmers, the remaining 21% living in Kilifi town [9]. Mosquito vectors are present year-round and residents receive from 1.5 to 8 infective bites per year in Kilifi town or the rural forest, respectively [41]. The parasite rate in healthy children is 34% averaged over the year, but transmission peaks after the rainy season when the parasite rate rises to 39%, remaining around 25% the rest of the year [9]. The clinical picture of malaria is typical of that in an endemic area in that clinical disease occurs primarily in children and malaria-specific mortality decreases with age, adults having acquired sufficient immunity to remain asymptomatic when infected. A minimum estimate of the incidence of severe malaria in children under 5 years is 14 per 1000 per year as estimated from hospital records [9]. The minimum estimate for the malaria-specific mortality rate in children under five years is 1.1 per 1000 children per year, but this is likely to be a significant underestimate because many deaths occur outside the hospital [9].

The data for this study were collected over a period of five years, from January 1989 to December 1993, by researchers from the Kenya Medical Research Institute (KEMRI) and by researchers from Oxford's John Radcliffe Hospital working in Kilifi. Our study population was derived from four distinct patient populations: KEMRI inpatients, pediatric ward patients, outpatient malaria cases, and healthy children from the community. All children included in the study had a primary diagnosis of malaria. Inpatients were from either the pediatric ward at Kilifi district hospital or the KEMRI ward, a five-bed pediatric intensive care unit constructed to facilitate research on severe pediatric malaria. The study included all patients admitted to either the KEMRI ward

or the hospital pediatric ward from January 1989 to December 1991, and additionally included all KEMRI patients admitted from January 1992 to December 1993.

Patients: Clinical Assessment and Laboratory Investigations

KEMRI patients were patients with severe malaria who were admitted to KEMRI ward either immediately upon presentation to the hospital or on transfer from the pediatric ward. Severe malaria was defined as having one or more of the following: severe anemia (hb < 5.1g/dL) with parasitemia ≥ 10,000/μL, prostration (unable to sit or stand), cerebral malaria (coma defined as child unable to localize to pain [42] more than one hour after a seizure and more than six hours after anticonvulsants), hyperparasitemia (>20% parasitemia), 2 or more convulsions within 24 hours before admission, and death with a diagnosis of malaria confirmed by blood film.

For the purpose of this study, children with severe malaria were classified as either cerebral or noncerebral malaria patients. Patients with severe malaria were considered noncerebral if they had a Blantyre coma score of 5. Cerebral malaria patients were those children who could not localize to pain. Cerebral malaria patients were divided into those who were cerebral on presentation (always cerebral) and those who became comatose during their hospitalization (new cerebral). Throughout this paper these two groups are analyzed together as cerebral malaria patients unless otherwise specified. A subset of those severe noncerebral patients with a hemoglobin less than 5.1g/dL were classified as anemic. Patients who were both anemic and cerebral were classified only as cerebral.

On admission a complete history was taken from the parent and a physical examination was performed. Multiple laboratory investigations and clinical assessments were performed on admission as data was often being collected for other investigative studies. Those data pertinent to this study include age in months, thick blood films, thin blood films for those with high parasitemias, hemoglobin, white blood cell count, red blood cell count, respiratory status (in respiratory distress, yes or

no) and coma status (using Blantyre coma scale) [9]. At least every six hours during the child's stay on the unit blood films were repeated and for those who were anemic or in coma, hemoglobin or coma score was rechecked, respectively. Outcome was recorded as dead, alive, or alive with neurologic sequelae.

Pediatric ward inpatients were those children who were admitted to the pediatric ward by the hospital staff for inpatient therapy. Data available on these patients consisted of age in months, admission blood film, blood count, and for some patients, hemoglobin and repeat blood films as indicated. Outcome was recorded as dead or alive.

The malaria outpatients were those who presented to the outpatient clinic at the hospital and were subsequently recruited as nonsevere case controls for another study [9]. Similarly, the sample of healthy children from the community was those children selected as "well" community controls for the same study, all of whom had blood films done. For these two groups of patients the child's age in months and the parasite density at the time of the encounter were recorded.

Data Analysis

For most analyses children were divided into groups according to their age in years:

Age 0 years = 0 to 11 months
Age 1 year = 12 to 23 months
Age 2 years = 24 to 35 months
Age 3 years = 36 to 47 months
Age 4 years = 48 to 59 months
Age 5 years ≥ 60 months

All children 6 years and older were included in the 5 year old age group because of small sample size and the relatively similar spectrum of malaria disease and relative "immunity" in these older children.

Parasite densities were calculated as explained previously. The number of parasites per 100 white blood cells was multiplied by the white cell count times ten.

The number of parasites per 500 red blood cells was multiplied by the red cell count times 2,000. If a corresponding blood count had not been obtained simultaneously with the blood film, the white or red cell count nearest in time to the blood film was used.

For the outpatient malaria cases a cut-off of 10,000 parasites/μL was established as a minimum cut-off for the diagnosis of clinical malaria in ambulatory children in an effort to exclude ill children with incidental parasitemias. The natural log of the parasite density (ln parasitemia) was used for all analyses involving mean parasite densities because of the right skew of the distribution of parasite densities. Admission parasitemias were used for all calculations except where specified. For all summary analyses "all inpatients" refers to all KEMRI and pedi ward patients from January 1989 to December 1991. Although KEMRI patients from 1992-1993 were also part of the data set, summary statistics for inpatients are best represented by the complete set of all inpatients from 1989-1991 as additional data in certain groups could skew the data. For comparisons with other groups, "all inpatients" refers to all KEMRI and pedi ward patients from 1989-1993.

For each group of patients the following statistics were calculated: mean age, mean natural log of parasite density, geometric mean parasite density and mortality. Mean parasitemias were compared between all possible pairs of groups. Each group was analyzed both as a whole and after stratifying by age in years. Correlations between parasitemia and age were assessed using linear regression.

Mortality for each group was compared with that of all other groups, stratifying either by age or by level of parasitemia. Analysis of variance was utilized to determine if mortality was affected by age, parasitemia or both. The parasitemia levels used were, in parasites per μL blood: level 1=0-9,999 parasites, level 2=10,000-49,999, level 3=50,000-99,999, level 4=100,000-499,999, level 5=500,000-999,999, and level 6≥1,000,000 parasites. Additionally, the geometric mean parasitemias and

mean ln parasitemias were compared for the group of patients who died versus those who survived.

Statistical Analysis

Statistical analysis was performed using SPSS for Windows (KEMRI Coastal Unit, Kilifi, Kenya) and STATA (Harvard School of Public Health). Multiple ANOVA and linear regression were used for analysis of relationships between age or mortality and parasitemia, t-test for unequal variances was used for comparison of mean parasitemias and mean ages, and Spearman's correlation was used in the analysis of some age/parasite relationships.

Terminology

For the purposes of this analysis, the following terminology will be used when referring to the various patient groups unless otherwise specified (in order of severity):

Cerebral ⇒ KEMRI inpatients with cerebral malaria at any time during admission

Always cerebral ⇒ KEMRI inpatients with cerebral malaria at time of admission

Newly cerebral ⇒ KEMRI inpatients with cerebral malaria which developed after admission

Noncerebral severe, or noncerebral KEMRI ⇒ KEMRI inpatients with severe noncerebral malaria

Severe ⇒ all KEMRI inpatients

Anemic ⇒ Those noncerebral KEMRI patients with hemoglobin < 5.1 g/dL

General pediatric ward, or pedi ward inpatients ⇒ inpatients on the pediatric ward with disease serious enough to require hospitalization but not necessarily meeting the requirements for severe disease

All inpatients ⇒ Inpatients from all of the above groups admitted between January 1989 and December 1991

Outpatients ⇒ Ambulatory children with malaria (parasitemia ≥ 10,000 parasites/μL and clinical signs of malaria)

Healthy/well community children ⇒ Healthy children who had blood films drawn for a previous study of parasite prevalence.

RESULTS

3281 children were studied of whom 2212 were inpatients, 438 were outpatients diagnosed with malaria, and 631 were healthy children in the community. Of the 2212 inpatients, 1676 had severe disease (admitted to KEMRI ward) and 536 had been admitted to the general pediatric ward. Of the 1676 KEMRI patients, 419 had cerebral malaria and the remaining 1257 had severe noncerebral malaria. 479 of the KEMRI patients had severe malarial anemia, and 407 of these were not obtunded (noncerebral).

Age Distribution

Ages ranged from 0 months to 157 months for all groups. The mean age for the entire study population was 28.2 months. The mean age for cerebral malaria patients was significantly higher than for anemic patients, 35.5 months versus 20.8 months ($p<0.0001$, see Figures 1 and 2). The mean age and age range for each group is shown below.

Group	Mean Age (months)	Median Age (months)	Range (months)
All children	28.2	23	0 to 157
Inpatients:	27.1	21	0 to 157
Cerebral	35.5	32	4 to 157
Severe Noncerebral	25.3	19	0 to 148
Noncerebral Nonanemic	27.6	21	0 to 148
Anemic	20.8	13	1 to 149
Noncerebral Anemic	20.5	12	1 to 121
Gen. Pedi Ward	24.7	19	0 to 127
Outpatients	28.8	25	2 to 90
Clinically well children	31.5	28	2 to 108

Parasite Densities and Prevalence

Among children diagnosed with malaria, parasite densities at time of admission to the hospital ranged from 0 to 3,339,600 parasites/μL (not all patients had parasites

on their first blood film after admission). Mean natural log parasite densities (mean ln parasitemias) and geometric mean parasitemias for each group were as follows:

Group	number of patients	mean ln para.	geomean parasitemia	min. para. (per μL)	max. para. (per μL)
all inpatients	1866	10.35	31,347	0	3,339,600
cerebral	419	10.48	35,562	0	3,339,600
noncerebral	1257	10.72	45,400	0	2,880,000
anemic	479	10.34	31,098	0	2,880,000
gen. pedi ward	536	9.80	18,092	0	999,999
outpatients	438	N/A*	N/A*	10,032	1,158,300
well children	631	2.5	12	0	50,406

* Not applicable because 10,000 parasites/μL cut-off was used

For the cross-section of 631 healthy children who had blood films done, the prevalence of parasitemia was 33%.

Relation Between Age and Parasitemia

For the analysis of the relationship between parasite density and age, age was analyzed using both age in months (a continuous variable) and age in years (analyzed as both a continuous and categorical variable). For the group of all inpatients, parasitemia was somewhat positively correlated with age (Spearman rank coefficient = 0.090, $p<0.0001$). This linear correlation held for either age in years or age in months as the explanatory variable. However, in analyzing any group on its own (cerebral, noncerebral anemic, or general pediatric ward patients) only noncerebrals and the subset of nonanemic noncerebrals showed statistically significant correlations between parasite density and age ($r=0.069$, $p=0.015$ and $r=0.103$, $p=0.003$, respectively, see Figure 3). This suggests that perhaps the slight correlation seen for all age groups was a result of combining different patient populations which had different mean parasitemias and different age distributions. For the outpatients, parasitemia was not correlated with age (Figure 4), nor did the healthy children demonstrate a trend toward lower parasite densities at older ages (Figure 5).

Although there was no correlation between parasite density and age for all severe cases taken together, when cerebral versus severe noncerebral disease were compared, cerebral malaria patients' parasitemias tended to decrease with age ($p=0.054$) as compared with the parasitemias of patients with severe noncerebral disease (Figure 6). For cerebral patients, then, we see a reversal of the direction of the trend of parasitemia with age seen for the group of all inpatients. Because of the slight correlation with age and the significantly different age distributions amongst the different groups of patients, further analyses were done after stratifying by, or otherwise controlling for, age.

Relation Between Parasite Density and Type or Severity of Disease

Cerebral disease versus other disease

The geometric mean parasitemias for cerebral versus noncerebral KEMRI malaria patients were not significantly different ($p=0.201$), nor were they different for cerebral patients when compared with all other inpatients on both the KEMRI and general pediatric wards (1989-1993). However, as mentioned above, the mean peripheral admission parasitemia decreased with age for cerebral malaria patients. Parasite density on admission was not significantly different for those children who presented in coma (not localizing to pain) versus those who presented unobtunded (Blantyre Coma Score=5) independent of age ($p=0.46$).

KEMRI inpatients versus others

The geometric mean parasitemia for KEMRI patients as a whole was significantly different from that for general pediatric inpatients ($p<0.00005$). Taken alone, both the cerebral and noncerebral KEMRI malaria groups had geometric mean parasitemias which were significantly different from those of the general pediatric ward patients. This was true for all ages taken together, but when analyzed by age in years, we see an effect of age in that the mean parasitemias are not significantly different for the three groups at the older ages, especially for the cerebral patients as compared to

the general pediatric ward patients (Figure 7). This is due to the decreasing parasite densities at older ages for the cerebral patients and slightly higher parasite densities for the 4 and 5-year-old general pediatric was patients.

Anemic patients versus others

When the anemic patients were separated from and compared to other KEMRI noncerebral patients who were not anemic, the anemic group had significantly lower parasitemias than did the nonanemic noncerebral patients ($p=0.0021$) however, the cerebral patient's mean parasitemia was not significantly different from that of the anemic patients ($p=0.4437$). Similar to the cerebral patients, the anemic patients demonstrated a trend toward lower parasitemias at older ages ($p=0.066$, Figure 8).

Inpatients (1989-1991) versus others

Inpatients had dramatically higher parasitemias when compared with outpatients, and an even greater difference was observed between inpatients and healthy children ($p<0.0001$).

Relation Between Mortality and Type or Severity of Disease

Mortality was significantly higher for the cerebral patients than for any other group (15.75%). Anemic patients had the second highest mortality rate (6.1%) as shown below. Mortality increased somewhat with parasitemia for each group except for malaria outpatients (Figure 9). Additionally, for all groups, parasitemia was on average higher for those children who died than for those who survived, but in no case was the difference statistically significant (Figures 10, 11 and 12).

Patient Group	Geometric Mean Parasitemia/µL Deaths	Geometric Mean Parasitemia/µL Survivors	Percent Mortality
Gen. Pedi. Ward	23,389	18,034	1.87
Noncerebral	81,634	44,356	3.18
Cerebral	51,534	33,190	15.75
Anemic	53,637	30,031	6.14
All Inpatients	48,533	30,638	4.61

Mortality was approximately constant over all ages for all inpatients taken together (Figure 13). Cerebral malaria patients had the highest mortality under 3 years of age, and all anemic patients who dies were under 3 years of age (Figure 12).

DISCUSSION

It has long been accepted that there is a correlation between parasite load and both severity of disease and outcome in *Plasmodium falciparum* malaria. What has not been clearly described is the nature of these relationships for different presentations of disease: cerebral malaria, severe anemia, and other presentations of variable severity. Our data support previous findings that there is an association between parasite density, as measured with a peripheral blood film at time of presentation, and severity of disease, but we have also found that for those presentations of malarial disease with the highest mortality (severe anemia, and particularly cerebral malaria) the association with parasite density is weakest. Thus, peripheral parasite density is not of prognostic value in predicting type or severity of disease or outcome for the individual patient. Furthermore, our data suggest that peripheral parasite density may not be of use as a surrogate measure of malaria-associated morbidity and mortality.

Parasitemia and Age in Severe Disease

Our data demonstrate a slight increase in peripheral parasitemia with increasing age, for all inpatients evaluated from 1989-1991, but this trend was small. This relationship was not significant for any group analyzed on its own.

There was a somewhat larger trend, of borderline significance, towards lower parasitemias at older ages in both the cerebral and the anemic patients ($p=0.054$ and $p=066$, respectively, Figure 6). While the relationship between parasite prevalence and age in malaria-endemic areas has been well studied, there has been little research into the relationship between parasite density and age. It has been well documented that adults from endemic areas tolerate higher parasitemias without disease than do

children, reflecting the development of immunity to malaria through repeated or even chronic exposure. The nature and scope of this immunity are poorly understood and there are several intriguing aspects to it. First, although the period of highest mortality from malaria is from 0 to 5 years of age, parasite prevalence continues to rise until age 10, when mortality dramatically declines [2]. Second, although immunity apparently builds over time, the average age of cerebral malaria patients is significantly higher than the average age of severely anemic patients [2, 7], 36 versus 21 months from our data.

One theory which attempts to explain the first observation, that of decreasing mortality in the face of increasing parasite prevalence, is the theory of strain-specific immunity [37]. The authors suggest that immunity to *P. falciparum* parasites is strain specific, and that immunity increases as children encounter each strain. Given a person with an asymptomatic parasitemia, the argument is that the quantitative effects of a new innoculum, i.e. the number of parasites contributed by a new bite at the beginning of the rainy season, would be far outweighed by the numbers of parasites contributed by multiplication of the preexisting parasites. Therefore, the authors propose that a qualitative difference in the new innoculum, rather than the addition of many more parasites, is what triggers disease. This theory is consistent with the observation that vector control through the use of bednets has reduced morbidity and mortality without reducing the prevalence of parasitemia. Although children are still parasitized, bednets may reduce morbidity by reducing the number of infective bites per year, because a lower infective bite rate most dramatically lowers the chances that a *less* common event, such as being infected by a new strain of falciparum parasite, will occur [6].

It is interesting to note that the age distributions for severe anemia and cerebral malaria are significantly different from one another. The mean age for cerebral malaria is 15 months older than that for severe malarial anemia in our study, and 18 months older in two other studies in The Gambia and Kenya [43, 8]. This suggests that the

pathophysiology of cerebral malaria is distinctly different from that of other forms of malaria in that the partial immunity which children develop over time does not confer the same protection from cerebral malaria as it does from other forms of disease. Perhaps previous exposure actually sensitizes an individual and is a prerequisite to the development of cerebral malaria [2]. Several other theories have been proposed to explain the observed age distribution.

It has been suggested that there may be rarer, more virulent strains of malaria which are more likely to cause cerebral malaria. In conjunction with the theory of strain specific immunity, this would suggest that while young children are continually challenged with the more common, less virulent strains, they do not develop protective partial immunity to the virulent strains because on average, the population's exposure to the virulent strains occurs at an older age. This theory would then implicate parasite virulence factors in cerebral malaria.

Parasite Virulence Factors in Cerebral Malaria

Some proposed parasite virulence factors include increased ability to induce tumor necrosis factor (TNF) production by the host, increased capability of binding to endothelial cells, increased ability to form rosettes, and variable rates of replication within the host [2, 7, 26, 34]. There is evidence to support some of these ideas. Extracts of some strains of parasites induce higher levels of TNF-α than do other strains [7], and higher levels of TNF have been found in the serum of patients with cerebral versus other forms of malaria [34]. Thus, TNF is implicated in cerebral disease and certain parasites are more capable than others of inducing TNF production. Also, parasitized red blood cells (PRBCs) from patients with cerebral malaria show varying degrees of binding to putative host cell receptors in vitro, suggesting that only some strains of P. falciparum express neoantigens capable of cerebral cytoadherence [44]. Additionally, some strains of *P. falciparum* do not produce knobs on the surface of the PRBCs. These knobless PRBCs do not bind to endothelial receptors such as

thrombospondin, suggesting that the ability to form knobs is another parasite virulence factor [4, 31].

Host Factors in Severe Disease

Host factors are also likely to influence the likelihood of cerebral malaria or other severe malaria disease. Variation in the host factors involved in cytoadherence may contribute to a person's "susceptibility" to cerebral malaria. That is, amongst individuals, differential expression of potential cerebral endothelial receptors for infected RBC neoantigens may result in differential "susceptibility". Those persons expressing more of the necessary receptors would experience cytoadherence, theoretically leading to gross RBC sequestration and thus cerebral malaria. TNF has been shown to upregulate I-CAM production, so a strain of parasite which was particularly effective at inducing TNF production could "uncover" a person's susceptibility to cerebral malaria by causing an upregulation of I-CAM or other putative receptors which would increase binding. Some people may be more susceptible to upregulation of this sort. Presumably, strain-specific immunity developed over time protects adults even if they are individuals "susceptible" to cerebral malaria.

Additionally, HLA type has been shown to play a protective role in malaria disease [29]. In The Gambia there is a high frequency of HLA class I antigen HLA-Bw53 which was associated with a 40% protection against both cerebral and severe anemic disease, and HLA class II antigen DRw13 was shown to be protective against severe disease but not cerebral malaria. That HLA class I antigens are protective against cerebral malaria but HLA class II antigens are not suggests that cytotoxic T-cell actions may be involved in the killing of pre-erythrocytic parasites whereas antibodies (produced in association with HLA class I antigens) may play a more important role in the prevention of severe disease other than cerebral malaria [2]. Clearly, the details of host-parasite interactions have not all been elucidated.

Mortality

Although mortality was higher in both anemic and cerebral disease than in other groups, the admission parasitemias of those who died were not significantly higher than the parasitemias of those who survived for any group (Figures 11 and 12). When all inpatients were considered together, those who died had a higher geometric mean peripheral parasitemia than those who did not die (Figure 10). Mortality increased in a linear fashion with increasing parasitemia for all KEMRI groups and for all inpatients, but there was not a significant linear trend for the general pediatric ward patients alone.

While peripheral parasite counts may be very useful in grossly determining who is at higher risk of death they are not useful for predicting clinical course or severity of disease, although it may be possible to establish cut-offs of parasite density within various categories of disease which may be of some prognostic value. For example, mortality is almost twice as high for all inpatients with parasitemias over 100,000/μL as for those with parasitemias less than 100,000/μL (7.96% versus 4.40%). Using a parasitemia cut-off of 100,000/μL produced the greatest difference in mortality between those above and those below the cut-off, so a parasitemia of 100,000/μL may be an appropriate cut-off as an indicator of increased risk of death. But as would be expected, clinical presentation seemed to correlate with outcome better than did peripheral parasite density. Mortality for all KEMRI patients was more than three times the mortality for general pediatric ward patients (6.32% versus 1.87%), and for cerebral patients mortality was more than eight times that for pediatric ward patients (15.75% versus 1.87%). None the less, the applicability of a cut-off "warning" parasitemia level as an indication for aggressive inpatient monitoring and parenteral treatment might merit further investigation.

Mortality did not vary dramatically with age for all inpatients considered together. However, cerebral malaria patients had the highest mortality for those under

2 years of age, and all of the deaths in the malarial anemia patients occurred under 3 years of age. This may be consistent with the theory of the development of partial strain specific immunity. If immunity is strain specific, then as people age, higher parasitemias may be tolerated without disease. If deaths occur at only a very young age, then we could postulate that this partial immunity protects not only against disease, but also against severe disease, and even mortality. Thus, when young children are exposed to strains which they have not previously encountered they are not only more likely to become sick than are older individuals, but they are also more likely to develop severe disease and to die. This conclusion is supported by the finding of higher mortality at younger ages.

Disease versus Parasitemia

Distinguishing between malaria parasitization and disease is not straightforward, and exactly what defines a "case of malaria" is a subtle point which is still disputed. Mortality in Kilifi District follows a pattern which is typical of endemic areas, being highest in the youngest age groups, when severe disease is most common [9]. But again, it is of interest to note that both the risk of severe disease and the risk of death reach their peak before the age at which the prevalence of parasites is highest. One explanation for this is that at younger ages parasitemia is more likely to cause disease, and that healthy children are healthy only because they are not parasitized, whereas at older ages parasitemia is better tolerated so that the prevalence of parasitemia in the healthy population increases. This has implications for the relationship between parasitemia and disease because while the partial immunity which develops over time is protective against disease it is apparently not protective against parasitemia. However, a cross-sectional study from western Kenya has suggested that immunity may reduce parasitemia too, in that healthy children had decreasing parasite densities over the ages 0 to 6 years.

In fact, it is not known how the partial immunity which develops through chronic exposure to malaria protects against disease without eliminating parasitemia. It may be, as discussed, that specific immunity is against parasite antigens which vary from strain to strain [39, 40]. It is also possible to imagine that what is being considered "partial immunity" is in part just an adaptation or a tolerance of the host to the effects of malaria parasitization. That is, the host does not manifest the symptoms of severe anemia (respiratory distress and heart failure) or does not, perhaps, mount the same immune response to parasite neoantigens, or the same cytokine response to waves of merozoites being released. This idea is consistent with the finding that immunity to disease is developed without complete immunity to infection.

In a survey study of healthy children in western Kenya with a mean parasite prevalence of 94.4%, a comparison of geometric mean parasite density and age revealed that there is a significant decrease in parasite density for older children as compared with younger ones (<2 years vs. 2-3 years vs. 4-6 years) [17]. If parasitemia declines with age, then the "partial immunity" which these children develop is actually reducing parasite density, not just disease. In this same study, prevalence of fever (defined as temp $\geq 37.5^{\circ}C$) was found to increase with increasing parasite density, and the authors suggest that parasite density could therefore be used as a surrogate measure of malaria morbidity and mortality. But fever alone in an ambulatory population represents the mildest form of disease. While parasite density may be an appropriate surrogate measure for mild disease and certain types of severe disease, and it may not necessarily follow that parasite density would be a good measure of mortality. In particular, it is not entirely clear whether the prevalence of fever is a reflection of the incidence of cerebral malaria disease and mortality as the authors suggest. This is because of the age distribution of cerebral malaria.

Whether the late age of onset of cerebral malaria is due to rare parasite strains or whether prior sensitization is required to trigger cerebral malaria, it is not apparent

how lower parasite densities in the population would confer any protection against this form of disease. That is, whatever it is about the immunology or pathogenesis of cerebral malaria which delays its onset relative to other forms of malaria disease, it seems likely that this age effect would preclude a direct correlation between parasite density in the population and cerebral malaria morbidity. If this were true, then the correlation between parasite density and mortality might be weakened because of the high proportion of malaria deaths due to cerebral disease.

Further Analyses to Consider

There are two factors which we did not assess in our study which would be of interest: history of antimalarial treatment prior to presentation at the hospital and stage of parasite development. We did not determine which patients had taken antimalarials prior to coming to hospital. Field and Niven found a correlation between mortality and parasitemia for those patients who had not taken chloroquine prior to the blood film. Perhaps including a history of treatment in the analysis would reveal stronger correlations in our data. Secondly, there is considerable evidence that a peripheral blood film with a predominance of late stage parasites is correlated with poor outcome and is suggestive of a greater load of sequestered parasites [12, 13, 14]. No attempt was made to assess parasite stage when the blood films for this study were read. But again, including parasite stage in our analysis would very likely help strengthen correlations or elucidate new correlations between parasite density and severity of disease.

Finally, a question to ponder: why is the spectrum of childhood disease so different in western versus coastal Kenya? Parasite prevalence is around 94% in western Kenya and severe anemia is the most common severe form of malaria, with cerebral malaria being relatively rare. In contrast, along the coast of Kenya parasite prevalence is around 34% and cerebral malaria and severe anemia are both seen, but cerebral malaria is far more common than in western Kenya. Could the differences in

spectrum of disease seen in these two areas be attributed to the difference in the force of transmission and the different parasite rates in the two areas?

Conclusion

The search for surrogate measures of malaria morbidity and mortality continues. Surrogate measures would facilitate evaluation of public health interventions in that labor intensive direct measurement of disease and mortality would be avoided. To date, no universal useful measure has been identified. Only one study has suggested that peripheral parasitemia measured in the general population may be of value in assessing the burden of malaria morbidity and may therefore be of use in evaluating the effect of interventions. Similarly, prognostic indicators applicable to individual patients are lacking. Peripheral parasitemia was shown long ago to have a general association with severity of disease and outcome. Further studies have attempted to elucidate combinations of clinical and laboratory assessments which would be of prognostic value in the evaluation of patients in hospital. Yet the fact remains that peripheral parasitemia alone is of virtually no prognostic value for the individual patient. Our data support these findings and further reveal that while severe disease is generally associated with higher parasitemias than is non-severe disease, those forms of disease with the highest mortality, cerebral malaria and severe malarial anemia, do not have significantly higher parasitemias than other severe malarial disease and in fact may have lower parasitemias. This reduces even further the usefulness of elevated parasitemia as a prognostic indicator of morbidity and mortality and also indicates that parasite density is perhaps only of secondary importance in, or is perhaps secondary to, the pathogenesis of severe disease.

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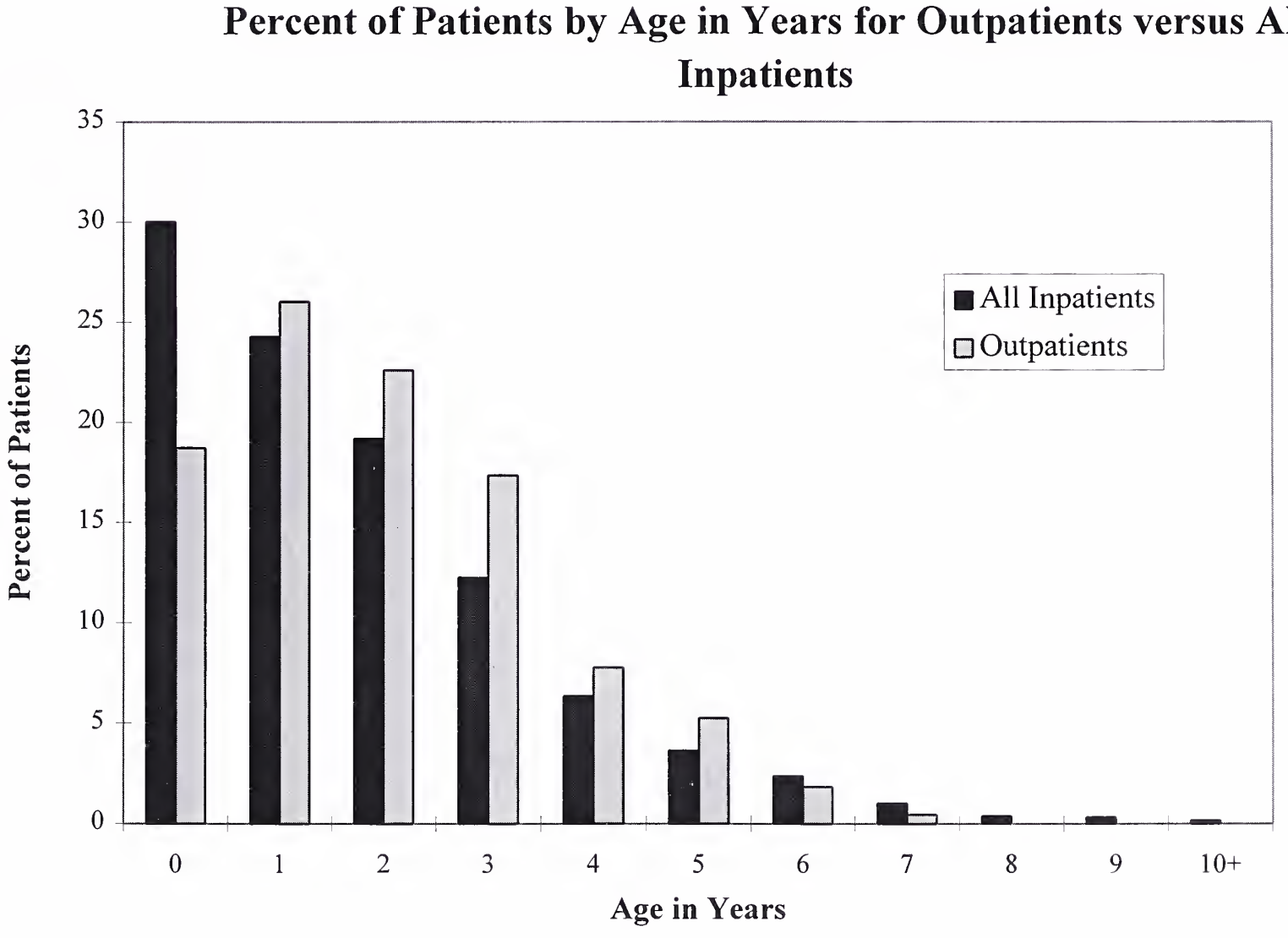

Percent of Patients by Age in Years for Outpatients versus All Inpatients
Percent of Patients
35
30
25
20
15
10
5
0
All Inpatients
Outpatients
0
1
2
3
4
5
6
7
8
9
10+
Age in Years

FIGURE 1

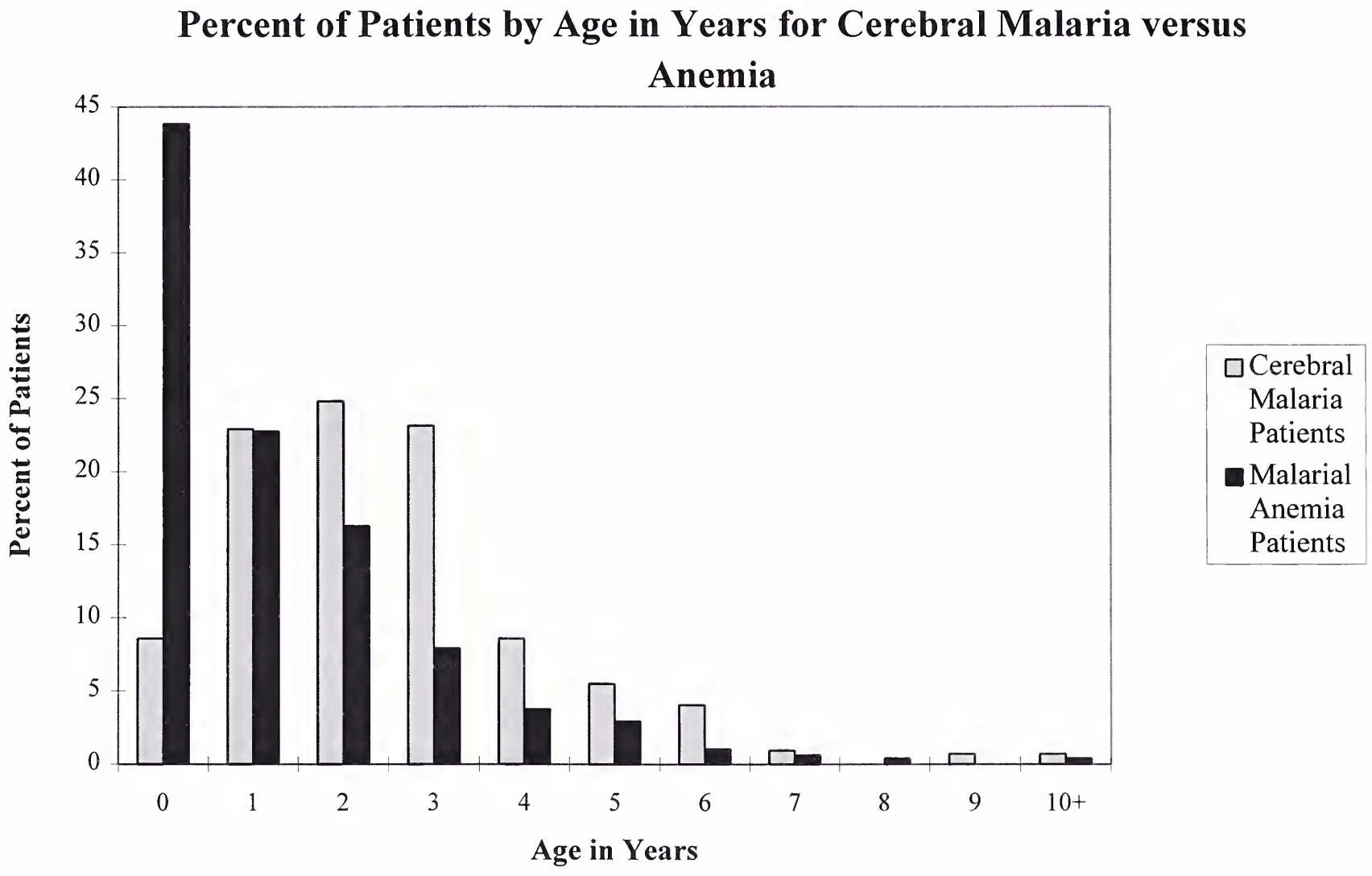

Percent of Patients by Age in Years for Cerebral Malaria versus Anemia
Percent of Patients
45
40
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
7
8
9
10+
Age in Years
Cerebral Malaria Patients
Malarial Anemia Patients

FIGURE 2

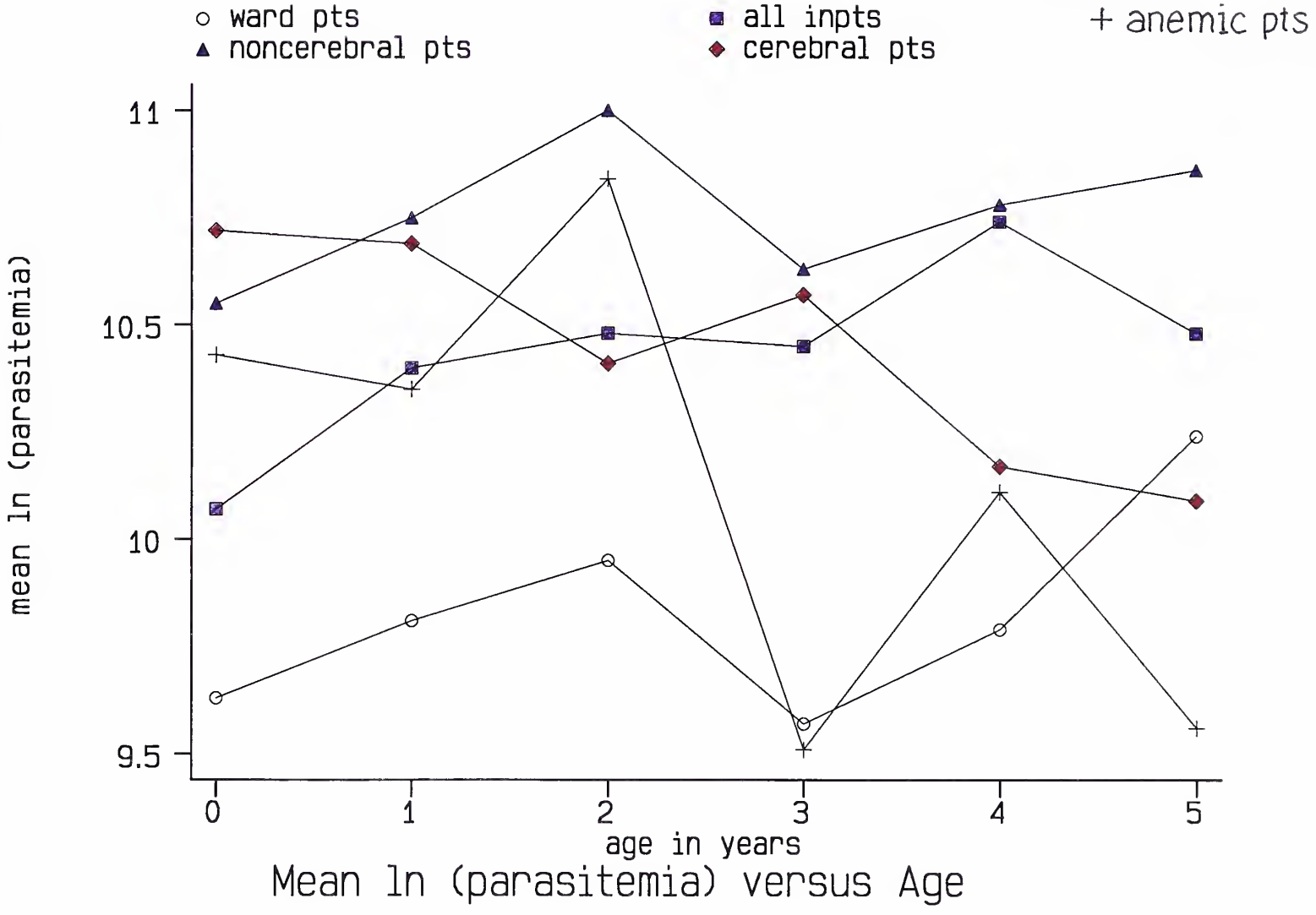

ward pts
noncerebral pts
all inpts
cerebral pts
+ anemic pts
11
10.5
10
9.5
mean ln (parasitemia)
0
1
2
3
4
5
age in years
Mean ln (parasitemia) versus Age

FIGURE 3

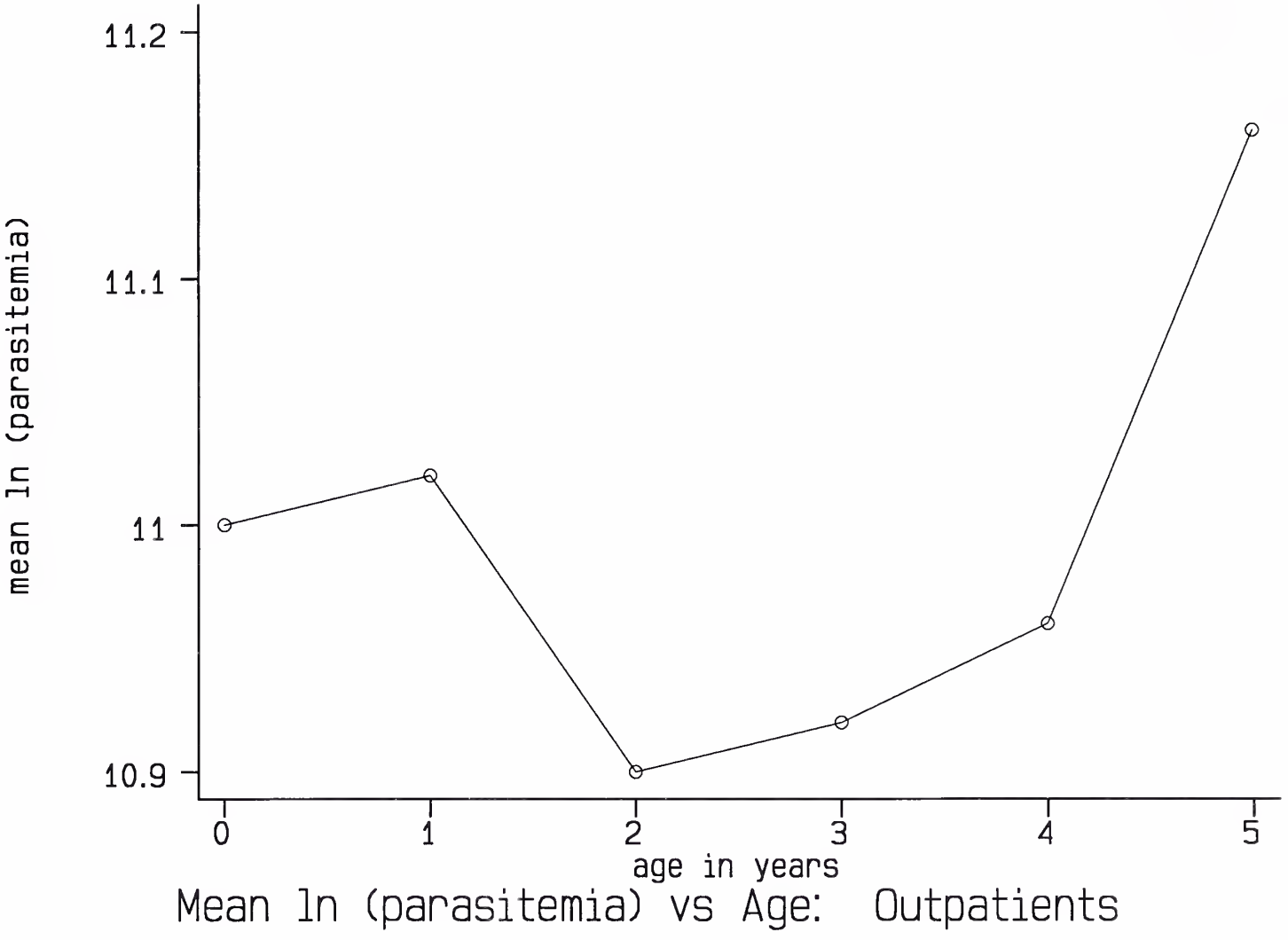
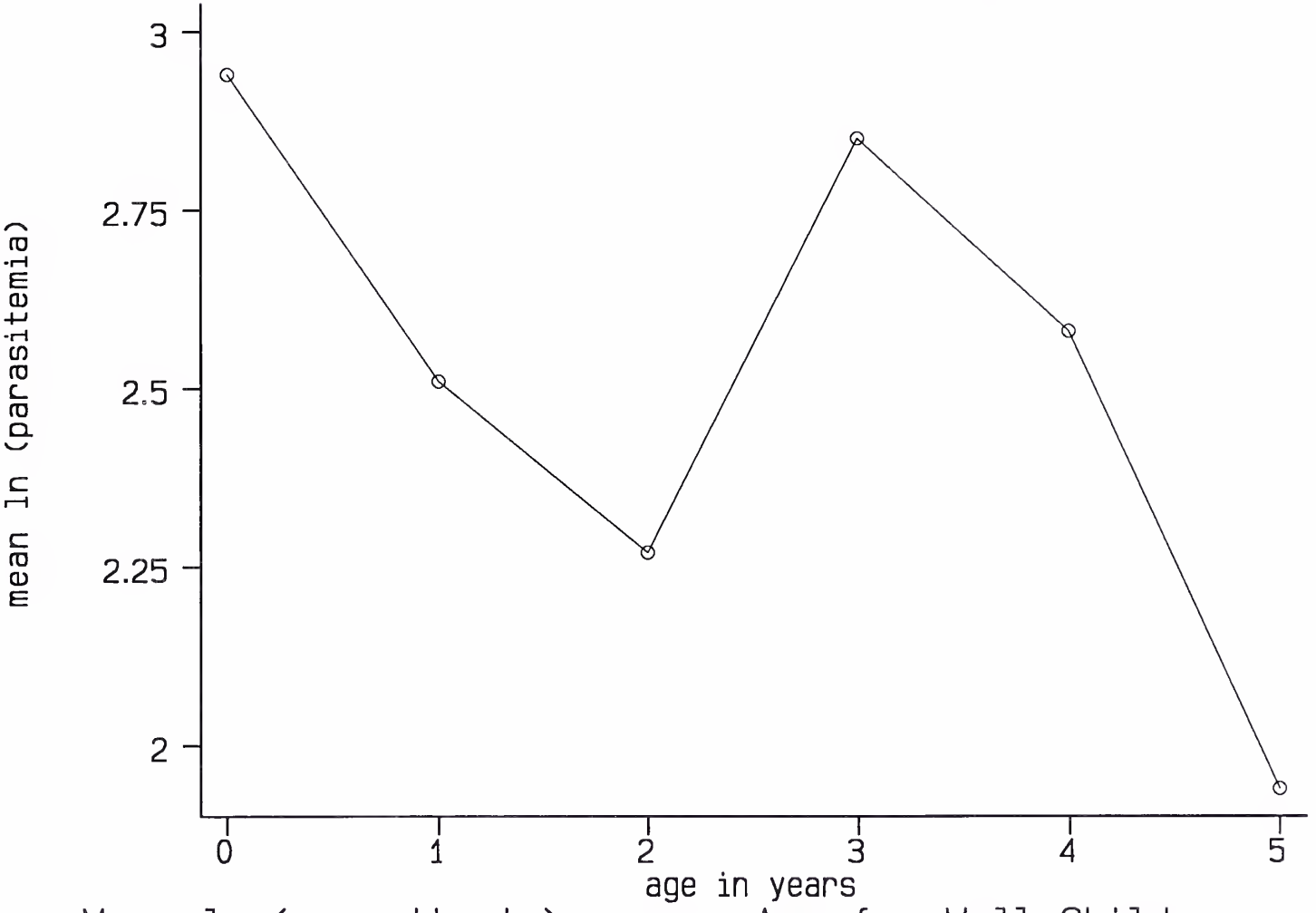
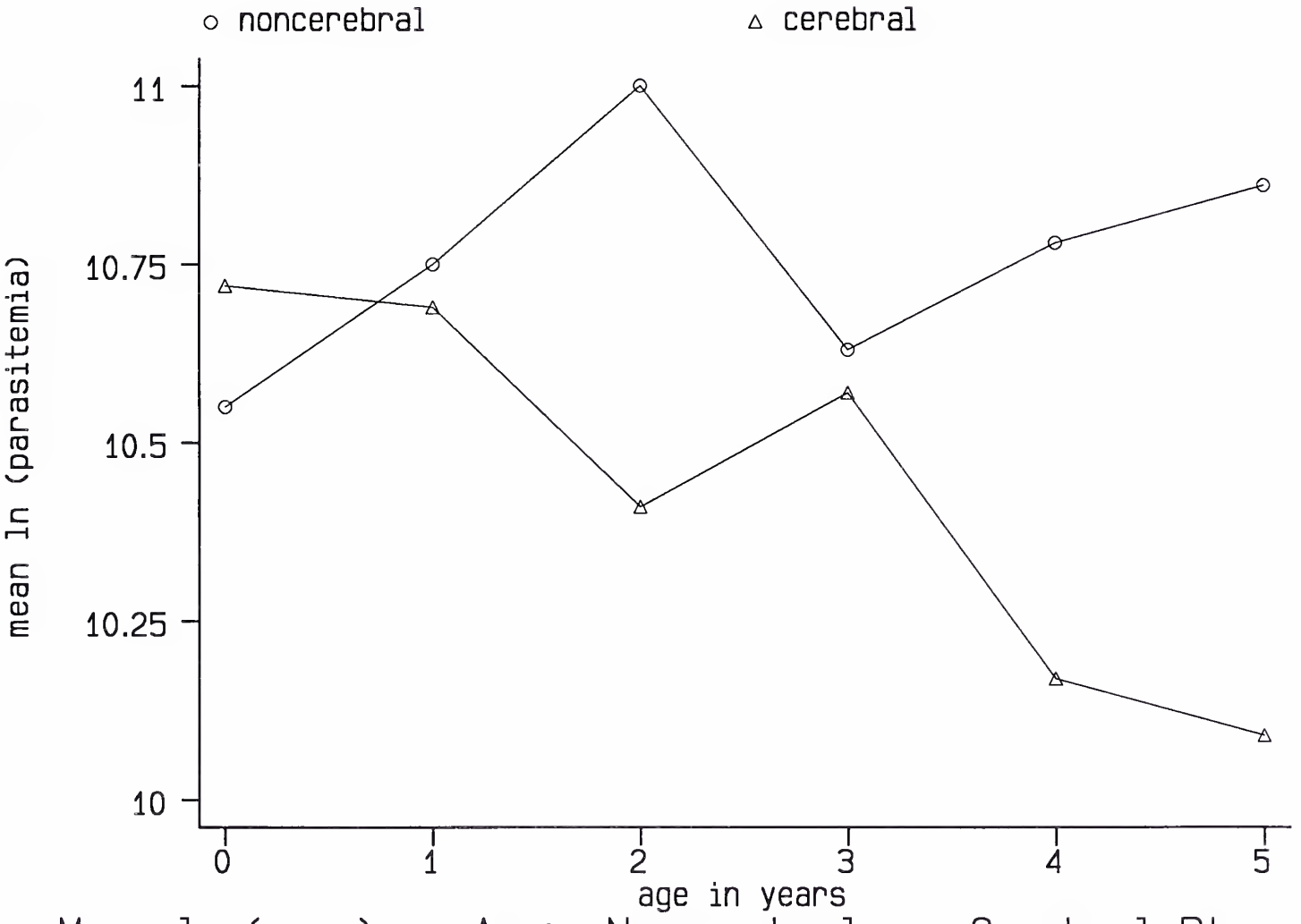

11.2
11.1
11
10.9
mean ln (parasitemia)
0
1
2
3
4
5
age in years
Mean ln (parasitemia) vs Age: Outpatients

FIGURE 4



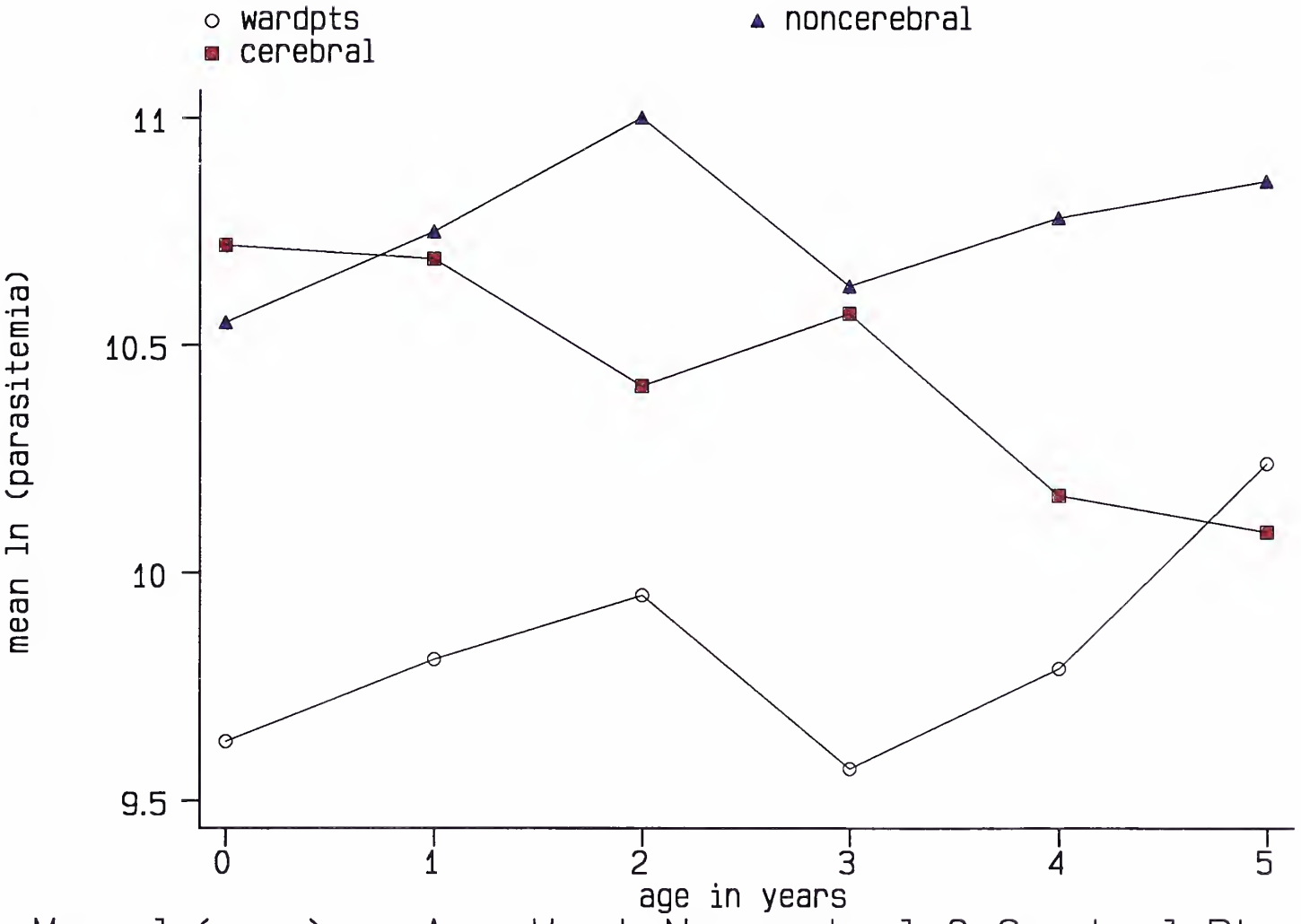
Mean ln (parasitemia) versus Age for Well Children

FIGURE 5



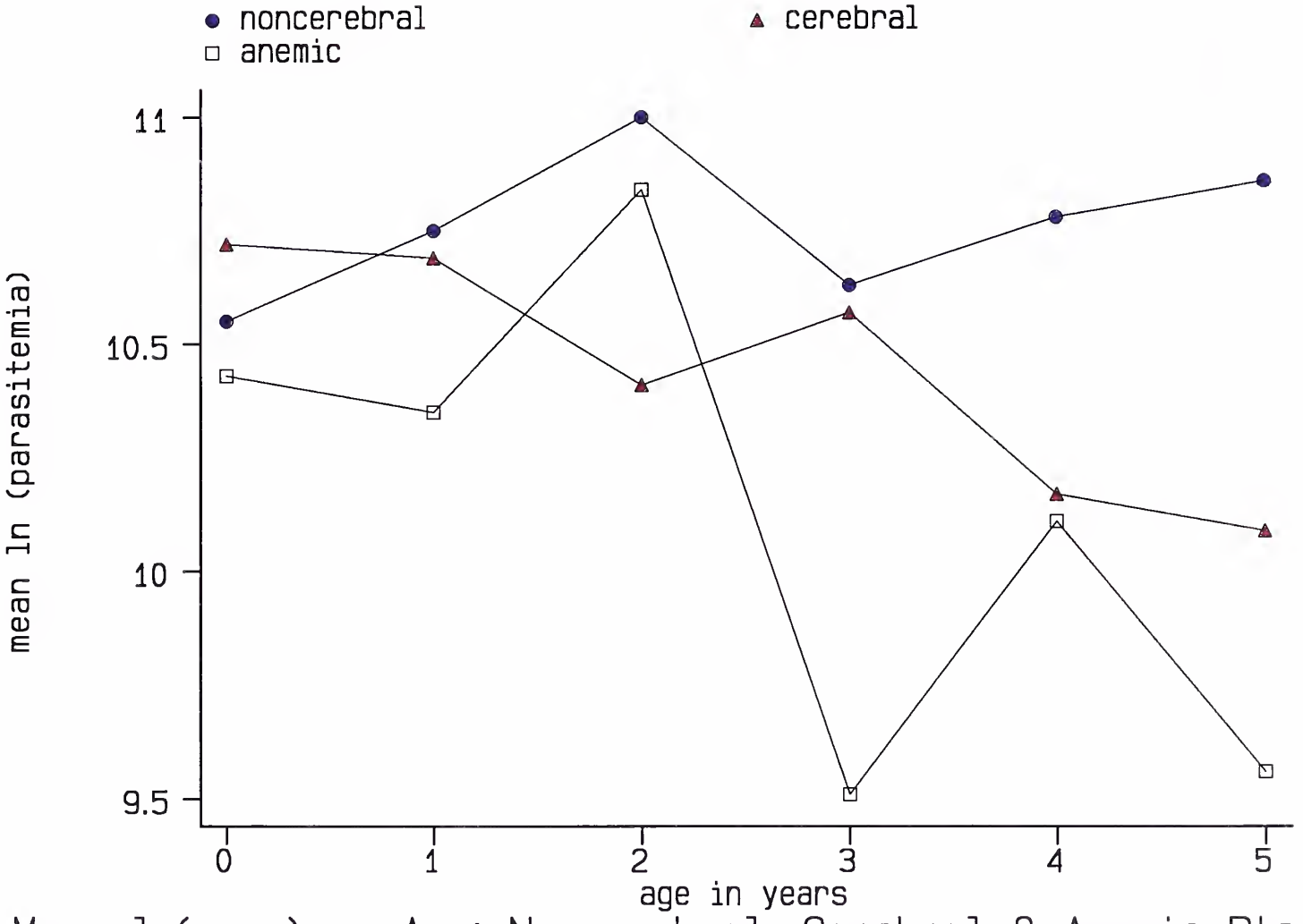
Mean ln (para) vs Age: Noncerebral vs. Cerebral Pts

FIGURE 6



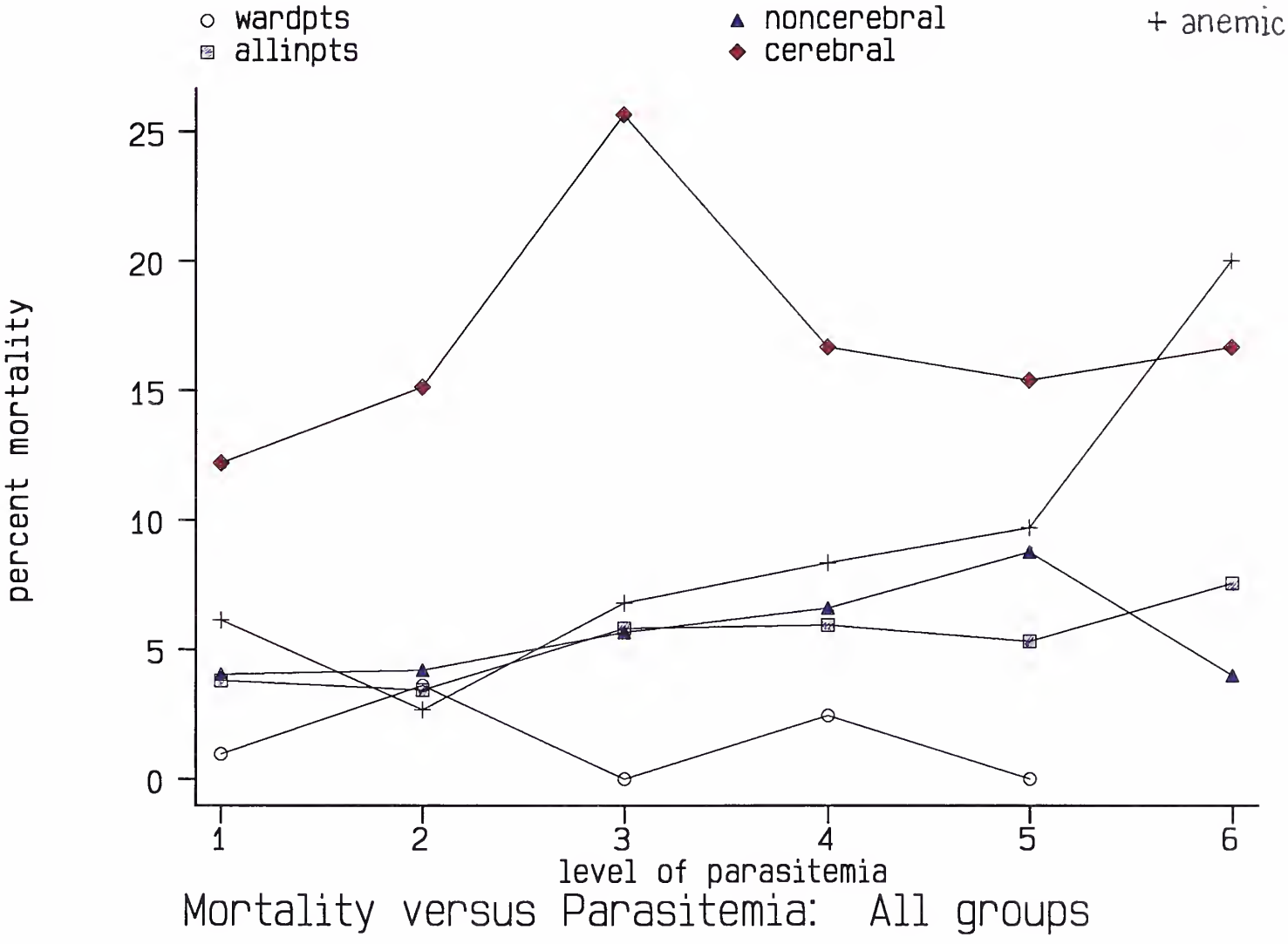
Mean ln(para) vs Age: Ward, Noncerebral & Cerebral Pts

FIGURE 7



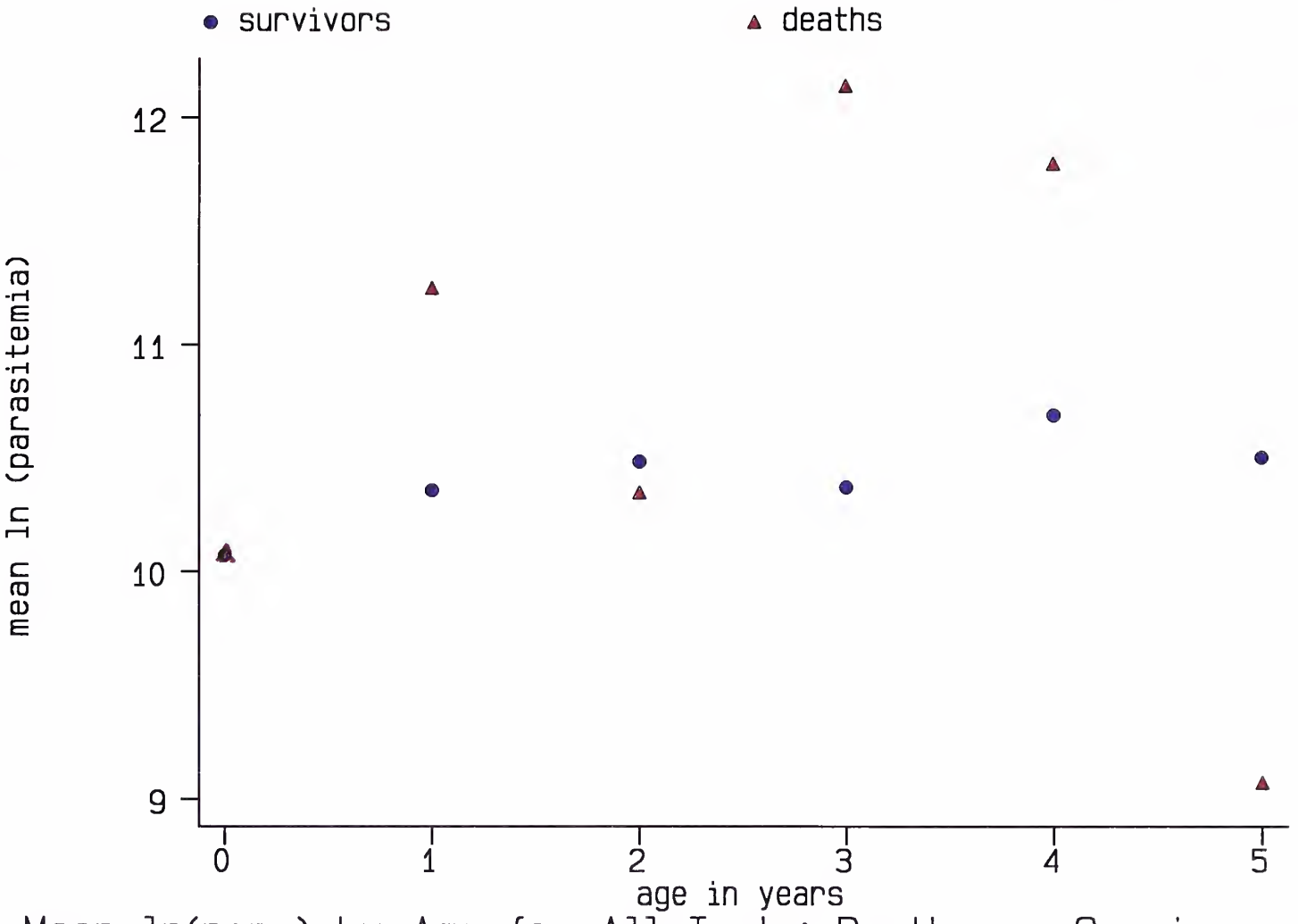
Mean ln(para) vs Age: Noncerebral, Cerebral & Anemic Pts

FIGURE 8



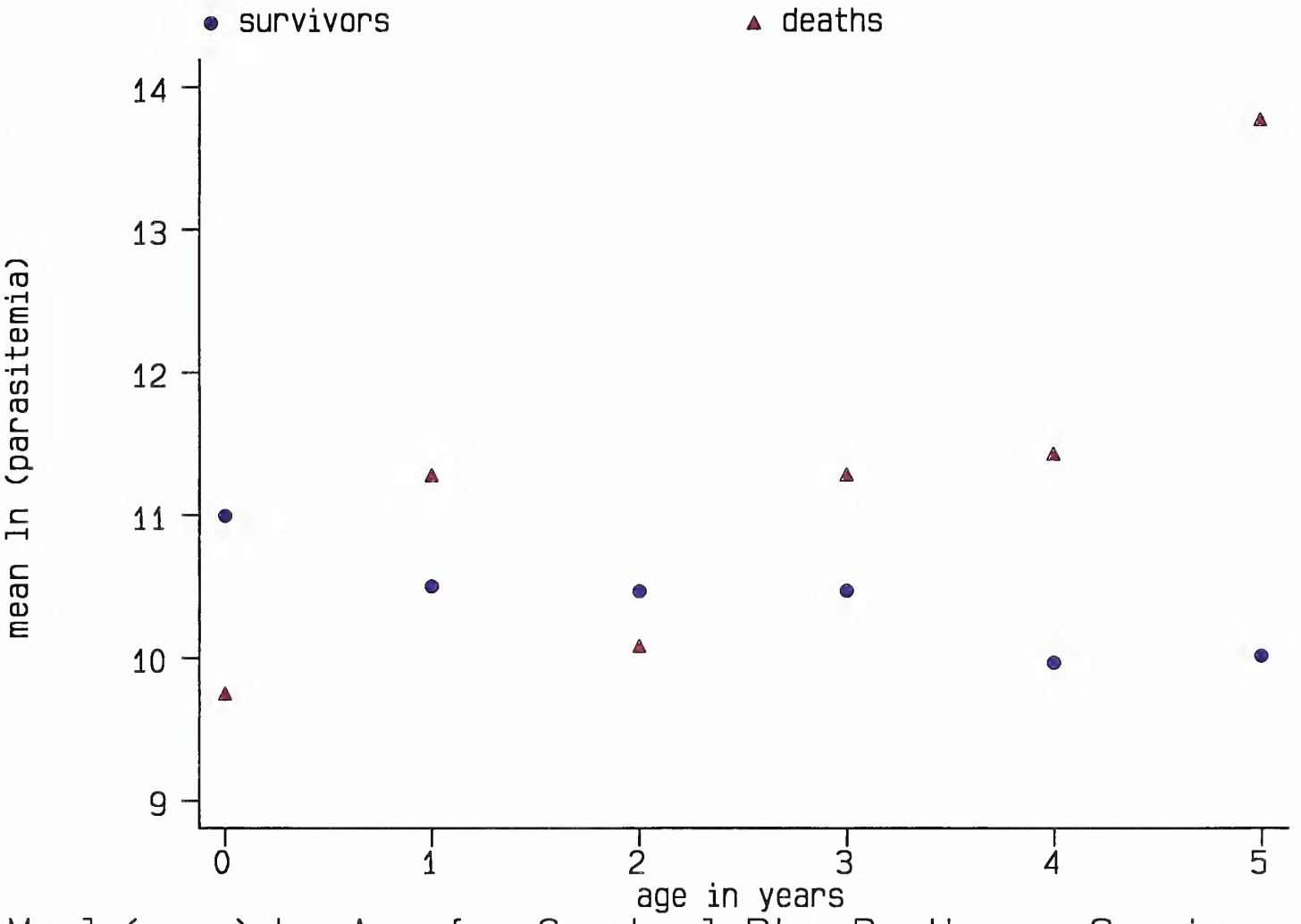
Mortality versus Parasitemia: All groups

FIGURE 9



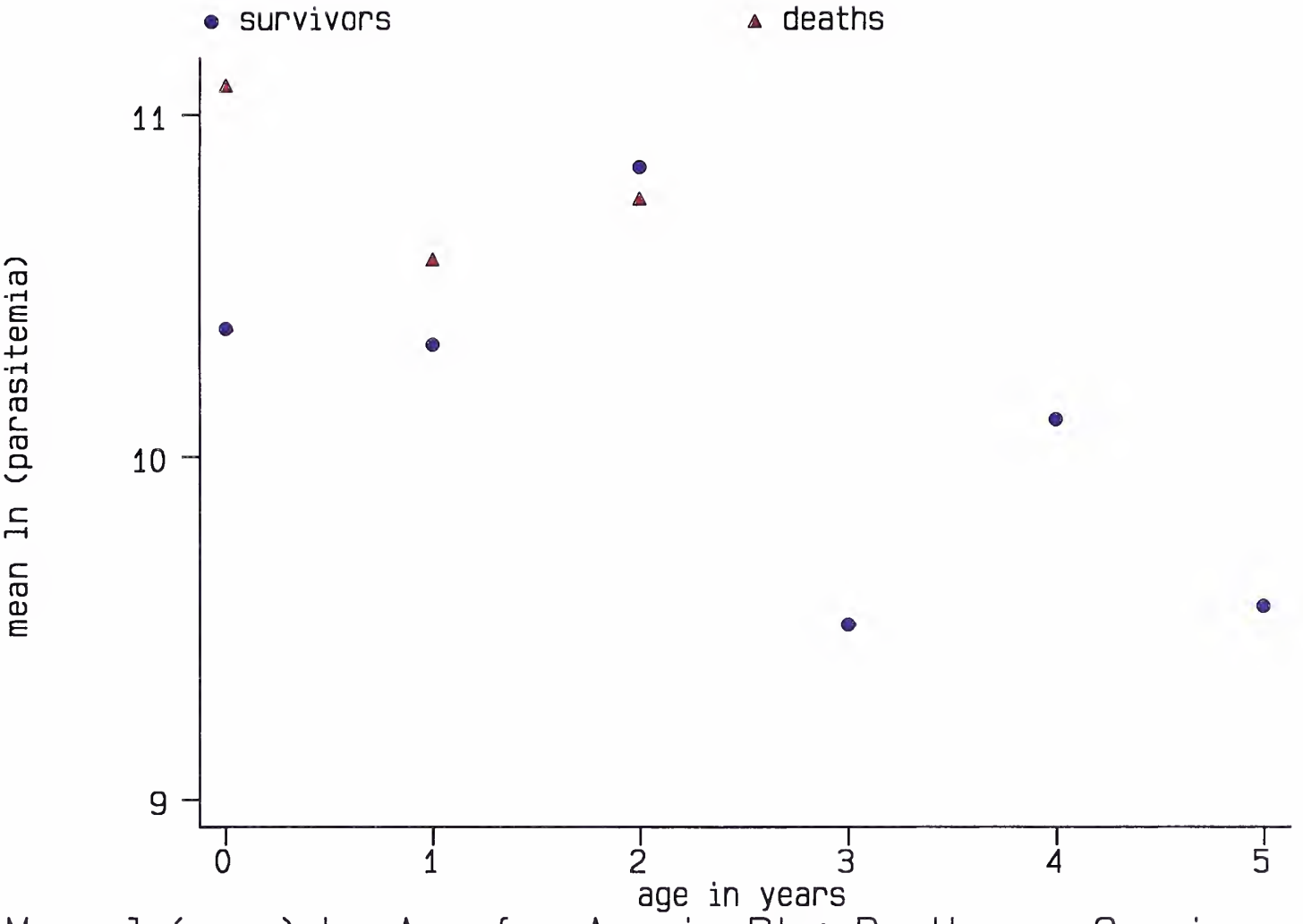
Mean ln(para) by Age for All Inpts: Deaths vs Survivors

FIGURE 10



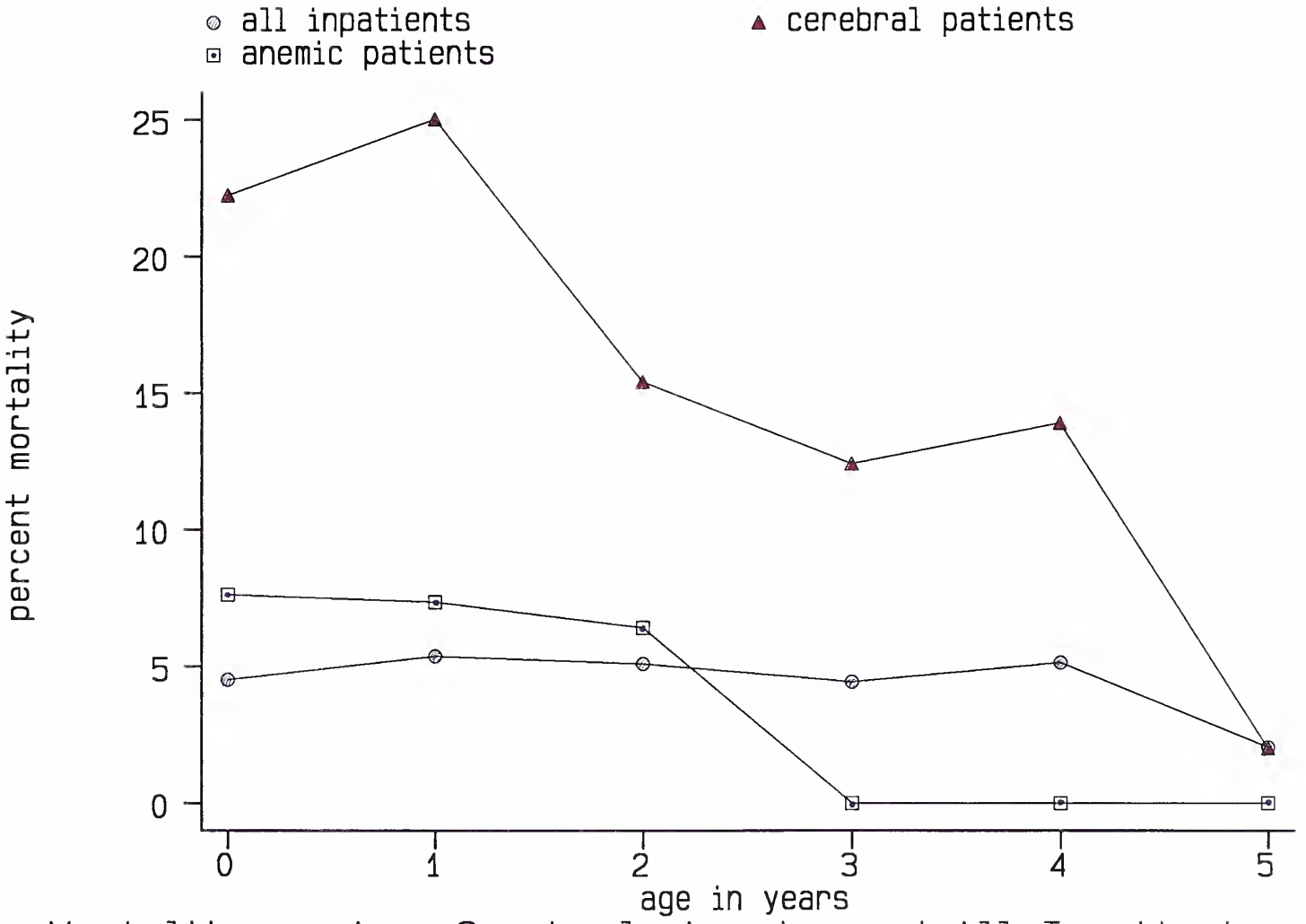
Mn ln(para) by Age for Cerebral Pts: Deaths vs Survivors

FIGURE 11



Mean ln(para) by Age for Anemic Pts: Deaths vs Survivors

FIGURE 12



Mortality vs Age: Cerebral, Anemic, and All Inpatients

FIGURE 13

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