Der Medizinischen Fakultät der Georg-August-Universität Göttingen

eingereicht von Prof. Dr. med. Uwe Groß

Establishment of a clinical algorithm for the diagnosis of *P. falciparum* malaria in children from an endemic area using a Classification and Regression Tree (CART) model

INAUGURAL – DISSERTATION

zur Erlangung des Doktorgrades

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List of abbreviations

AA	Artesunate/Amodiaquine
ACTs	Artemisine-combination therapies
AL	Artemether-Lumefantrine
APH	Agogo Presbyterian Hospital
BNITM	Berhard Nocht Institute for Tropical Medicine
CART	Classification and Regression Tree
CBHI	Community-based health insurance schemes
CI	Confidence interval
CRF	Case report form
EBT	Elevated body temperature
EDTA	Ethylenediaminetetraacetic acid
EPO	Erythropoetin
GCP	Good clinical practice
GHS	Ghana Health Service
Hb	Haemoglobin
ICF	Informed consent form
IMCI	Integrated Management for Childhood Illness
IPTI	Intermittent Preventive Treatment for infants
IPTP	Intermittent Preventive Treatment in pregnancy
KNUST	Kwame Nkrumah University of Sience and Technology
LLINs	Long-lasting insecticidal nets
LR	Likelihood ratio
NPV	Negative predictive value
NRS	No respiratory symptoms
OPD	Outpatient department
OR	Odds ratio
P. falciparum	*Plasmodium falciparum*
PPV	Positive predictive value
RDT	Rapid diagnostic test
ROF	Report of fever

RTI	Respiratory tract infection
SDs	Standard deviations
SP	Sulphadoxine-Pyrimethamine
SSA	Sub-Saharan Africa
UNICEF	United Nations International Children's Emergency Fund
URTI	Upper respiratory tract infection
UTI	Urinary tract infection
WHO	World Health Organization

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1.Introduction

Despite recent reports of declining prevalences of malaria infections in most parts of the world, the disease remains one of the most frequent causes for death in children under five years of age in Sub-Saharan Africa. Especially children living in countries that are holoendemic for malaria, with high parasite transmission during the whole year are inescapable exposed to the parasite vector *Anopheles*. As severe courses of *Plasmodium falciparum* malaria are common in children, diagnostic and treatment should be quick and appropriate. But by the way of contrast patients with suspected malaria are often rather treated presumptively as the health infrastructure lacks in laboratory equipment. To prevent missing sick children in poor medical settings and to avoid over-treatment, diagnostic algorithms could be a basis for evidence-based decision making.

1.1 Morbidity and Mortality of children in Africa

Every year ten million children under five years of age die worldwide due to diseases that could have been treated with medication or prevented by a vaccination. The highest death tolls for children are documented for the African continent, whereby the risk of death is the highest in the first month of life. Fourty-three percent of infant mortality cases are found in the neonatal period (≤ 28 days of life). The main causes for death are age-dependent. While perinatal infections, early births, low birth weight, asphyxia and birth trauma account for the majority of fatalities in the neonatal period, in the postnatal period respiratory infections, malaria and diarrhoeal diseases are to the fore (World Health Organization 2012a).

For the "World Health Organization (WHO) Millennium development goal (MDG) region Sub-saharan Africa", the 'under-5 mortality rate' was 109 per 1000 live births in the year 2011. In Ghana figures were lower with 78 per 1000 live births, but still above the global average of 51 per 1000 live births (World Health Organization 2012a). The United Nations International Children's Emergency Fund (UNICEF) ranked Ghana's under-5 mortality rate on position 46 out of 193 countries in 2008, whereby position one reflects the highest under-5 mortality rate (Unicef 2010). In most developing countries, more than two thirds of events of deaths are due to five common diseases or conditions: Respiratory infections, malaria, diarrhoea, malnutrition and measles (World Health Organization and Ghana Office 2004).In

Ghana Malaria accounts for 26% of deaths in children under five years of age in 2008, followed by prematurity with 12 %, pneumonia with 10% and birth asphyxia with 11% (World Health Organization 2010a).

Malaria is known as a major cause of death in African children for a long time and is still liable for one in five death cases on the African continent. Each year 300 to 500 million people worldwide become infected with malaria parasites, more than 75% of these infections occur in African children under five years of age (Roll Back Malaria Programme 2004). However, in the last decade there is increasing evidence that the amount of malarial infections in sub Saharan Africa decreases (Guerra et al. 2008, Nyarango et al. 2006, Okiro et al. 2007, Sharp et al. 2007).

1.2 Malaria

Malaria is a protozoan infection of red blood cells transmitted by the bite of a blood-feeding female anopheline mosquito, in Africa usually *Anopheles gambiae* or *A. funestus* (Cook and Zumla 2009a). Of the over 200 known plasmodium species five are recognized to be pathogenic to humans as malaria parasites. The four most common species of human malaria parasites are *P. vivax*, *P. ovale*, *P. malariae* and *P. falciparum*, which has the greatest impact in terms of mortality. Recently,*P. knowlesi* has also been found to be able to infect humans (Jongwutiwes et al. 2011, Khim et al. 2011). The life cycle of *Plasmodium* is complex, as the parasite needs two hosts, the mosquito vector and a vertebrate host. In the human host, the cycle comprises of a liver- and blood stage and involves several phases of maturing of the parasite. While most of the parasites asexually replicate in the blood vessels, a small fraction differentiates into male or female sexual forms, which can be taken up by a female mosquito, undergo sexual reproduction in the midgut of the anopheles (Cook and Zumla 2009b).

According to estimations of the United Nations, young children in the age of 5 years or below from holoendemic areas in Africa have between 1.6 and 5.4 episodes of malarial fever each year (Murphy and Breman 2001). In highly endemic areas up to 70% of the one-year-old children have malaria parasites in their blood (Roll Back Malaria Programme 2004).

In an epidemiological survey in central Ghana in the year 2009, similar figures were found with a parasite prevalence of 64% and a maximum of seven malaria attacks per child per year in children below five years of age (Owusu-Agyei et al. 2009).

Especially in areas of stable malaria transmission, acquired immunity is an important factor that prevents older children and adults from severe courses of malaria. Maternal antibodies usually protect neonates for the first six months of life, after which they become more susceptible to the parasite and develop more severe courses. Although the frequency of clinical malaria and the risk of death begin to decrease in children 2 to 5 years of age, full protection rarely occurs before the age of 5 years. In areas of uninterrupted high transmission, stable protection from severe disease is usually acquired from puberty onwards. Afterwards malaria episodes still occur, but less frequent and usually of mild character (Doolan et al. 2009).

1.3 Clinical features of Malaria

Clinical symptoms of patients with malaria are various and alternate depending on whether partial immunity is already achieved. Patients with transplacental developed protection or acquired partial immunity generally show only moderate physical impairment often initiated by a prodromal stage of tiredness and aching.

Classical features for non-immune patients are an abrupt onset of an initial 'cold stage' with, sometimes heavy, rigors, a subsequent 'hot stage' with fever and a 'sweating stage', when the patient's temperature returns to physiological values. Those fever cycles are classically following a specific periodicity with asymptomatic periods, depending on the infecting *Plasmodium* species.

In *P. falciparum* malaria, there is often no obvious periodicity perceptible, since fever may be continuous and accompanying symptoms like myalgia, headache and vomiting may be permanent. *Plasmodium falciparum* involves the threat of inducing severe courses of malaria particularly in young children and non-immune adults.

Severe malaria is defined as the result of extensive multi-system involvement in *P. falciparum* malaria. Particularly in children, malarial infections tend to progress rapidly and death possibly occurs within hours. Neurological signs, like diffuse cerebral dysfunction with coma or convulsions may be signs of development of a cerebral malaria, which is one of the most important complications (Cook and Zumla 2009c).

The majority of children who are brought to outpatient departments in life-threatening conditions, who die, die within the first 24 hours of admission (Marsh et al. 1995). This implies that proper diagnosis and early treatment is essential and life saving.

In the past, several studies have been conducted to describe clinical features of major life-threatening conditions (e.g. malaria, sepsis, etc.). The findings should provide evidence-based data for clinicians to enable them to come to a treatment-decision, even without laboratory or technical support (Dzeing-Ella et al. 2005, Marsh et al. 1995). The subjacent aim of this attempt was to objectify classification of severely ill children, which is yet widely subject to individual knowledge and experience of the treating physician.

According to earlier studies, elevated body temperature or a report of fever, digestive disorders and convulsions represent some of the independent predictors for an infection with malaria (Bojang et al. 2000, Chandramohan et al. 2001, Muhe et al. 1999). Researchers in Niger (Gay-Andrieu et al. 2005) could also show that neurological symptoms like coma or impaired consciousness were significantly more often presented by patients with malarial parasitaemia than by those children without parasites in their blood. Chandramohan et al. (Chandramohan et al. 2002) showed correlation of absence of cough, presence of pallor and a palpable spleen with a malaria infection.

A study with Gabonese children depicted that hyperglycaemia and hyperlactataemia, occurring in 10% and 16% of the children respectively, were frequent laboratory features in patients with malaria (Dzeing-Ella et al. 2005).

1.4 Diagnostic tools and treatment of malaria

For decades microscopic detection of malaria parasites has been the gold-standard as diagnostic criterion for anti-malarial treatment as it still is (World Health Organization 2010b). But with the development of reliable alternatives such as rapid diagnostic tests (RDT's), limitations of malaria parasite microscopy known already, got in the focus of discussion. Comparative studies revealed poor quality of microscopic examinations (Mukadi et al. 2011) particularly due to a lack of training of health staff (Bell et al. 2005, Bloland et al. 2003, Moerman et al. 2003) resulting in frequent overdiagnosis and overtreatment especially in lower level health facilities (Kahama-Maro et al. 2011). Especially in those settings RDT's have been proven to be a reliable option beside microscopy (Moody 2002, Murray et al. 2003, Ochola et

al. 2006, World Health Organization 2003) for improving the management of febrile patients (Reyburn et al. 2007). Although RDT's do not generally give reliable results in case of patients with very low parasite densities (≤200/µl) (McMorrow et al. 2011), in high-transmission settings they can reduce prescription of anti-malarial drugs and prevent over-treatment (Ishengoma et al. 2011, Msellem et al. 2009). Also, they are effectively applicable by community health workers (Chanda et al. 2011, Mubi et al. 2011).

Development and distribution of RDT's was focused by the WHO, as the recommendation for treatment with new Artemisine - combination therapies (ACT's) led to expanding costs (Mutabingwa et al. 2005). Authorities were forced to seek for less complicated and cheaper methods than microscopy to detect focus groups that are to be treated with anti-malarials. In the year 2010 a total of 16 African countries were deploying RDT's at the community level while the number of microscopic examinations fell about 10% from 2005 to 2009 (World Health Organization 2010b). Studies concerning the clinical implementation of RDTs showed that there are still clinicians who are not willing to consider parasite-test results for their treatment decision (Batwala et al. 2011, Lubell et al. 2008, Reyburn et al. 2007). Since 2010 the WHO recommends presumptive treatment only for febrile children under five years of age living in high transmission areas. The organization therewith moved towards a parasite-based diagnosis in areas with medium and low transmission (World Health Organization 2010b), where decision-making was still based on subjective assessment of clinical signs and symptoms (Reyburn et al. 2007, Zurovac et al. 2006b).

After evidence was growing that resistance to commonly used monotherapies, i.e. Chloroquine and Sulphadoxine-Pyrimethamine does not guarantee reliable success in treatment of *P.falciparum* malaria any longer, the switch to Artemisine-based combination therapies (ACT's) was made in the early decade of 2000 (Bjorkman and Bhattarai 2005, Snow et al. 2005). By the end of 2009 all 42 african countries that were endemic for *P. falciparum* used ACT's, whereby Arthemether – Lumefantrine (AL) accounted for the largest proportion of sold ACT's followed by Artesunate – Amodiaquine (AA) (World Health Organization 2010b). Only sparse data is available concerning the question if the amount of distributed anti-malarials meets the needs of the patients, but it was however estimated that an average of 65% of treatment needs are fulfilled (World Health Organization 2010b).

Apart from standard treatment, 'Intermittent Preventive Treatment in pregnancy' (IPTp) provides pregnant women with a single dose of Sulfadoxine – Pyrimethamine (SP) at the beginning of the second trimester, followed by at least one dose no less than one month later (World Health Organization Technical Expert Group 2007). The effect of IPTp is described to depend on the susceptibility of the parasite to SP (Peters et al. 2007) and ideally comprises reduction of the prevalence of placental malaria, maternal anaemia and low birth weight (Kayentao et al. 2005, Peters et al. 2007). However evidence of IPTp – failure also exists (Gies et al. 2008, Hommerich et al. 2007, Parise et al. 1998, Verhoeff et al. 1998) and is mainly attributed to accumulating resistance to SP (Harrington et al. 2011). In 2009, 33 of 43 endemic African countries have adopted IPTp - strategies (World Health Organization 2010b). Another preventive model targeting children under five years of age is the 'Intermittent Preventive Treatment for infants' (IPTi) which involves provision of single dose Sulphadoxine – Pyrimethamine in parallel when they receive routine vaccinations for three times. Although not yet implemented in the WHO guidelines, research on IPTi – effectiveness delivered promising results (Aponte et al. 2009, Gosling et al. 2009, Grobusch et al. 2007, Manzi et al. 2008, Willey et al. 2011).

1.5 Clinical algorithms for diagnosing malaria

In the early nineties of the last millennium clinicians in malaria endemic countries were facing the situation of increasing death tolls due to malaria, while laboratory opportunities for confirmation of *Plasmodium* parasites were broadly lacking and medication was not yet widely enough distributed to cover all patients at need.

With the first attempts of generating algorithms for clinical decision-making, researchers targeted the question, if it would be possible to distinguish on a clinical basis between children who are suffering from malarial parasitaemia and those who suffer from different conditions (Redd et al. 1996, Rougemont et al. 1991). To this point most children presenting to a health facility with fever or a history of fever were presumptively treated for malaria. This attempt, which is even recommended today by the WHO for some settings (World Health Organization 2010b), ensures a maximum of sensitivity to ensure capturing all parasitaemic children.

In the following years researchers developed algorithms and case definitions in different settings and presented various results (Genton et al. 1994, Olivar et al. 1991, Schellenberg et al. 1994). While a study from Zimbabwe could not find any

significant associations between certain symptoms and parasitaemia (Bassett et al. 1991), others delivered a combination of symptoms that were able to increase the specificity of the diagnosis (Muhe et al. 1999). To compare the predictive value of an algorithm, performance of medical professionals using their clinical skills alone was set as a benchmark. In studies from Gambia and Tanzania pediatricians achieved this with a sensitivity of 86% and 99% and a specificity of 61% and 52%, respectively (Olaleye et al. 1998, Rooth and Bjorkman 1992). Researchers in Niger calculated a specificity for health worker diagnosis of 21% in the dry season and 0% in the wet season (Olivar et al. 1991). However, comparability of a study from the Sahel zone to those from Sub-Saharan Africa with stable transmission may be limited.

In 1998, Olaleye et al. identified four symptoms (reduced feeding, sleepiness, shivering and cough absent) and five signs (feeling hot, palmar pallor, rash absent, cough not heard and increased respiratory rate) as promising predictors for malaria. After developing a score-based algorithm using a simple count of signs and symptoms present in the assessed child and by applying a cut-off of 6 or 8 symptoms respectively, a sensitivity of up to 90% and a specificity of over 60% was obtained for the diagnosis of malaria (Olaleye et al. 1998). Two years later, Bojang et al. used this algorithm for a prospective evaluation in Gambian children to test its validity and ease of use. Tested in a small population of 382 children, the application of this algorithm with an optimal cut-off score resulted in a sensitivity of 88% and a specificity of 62% for a diagnosis of malaria. For the same population sensitivity and specificity were obtained for a physician's diagnosis without laboratory results, with 82% and 61% respectively (Bojang et al. 2000).

Rougemont et al. 1991 stated in their policy paper a case definition of a rectal temperature >37.7° C or splenomegaly or nailbed pallor would be able to increase specificity of the diagnosis to 41%, while sensitivity remains high with 85% (Rougemont et al. 1991). In a more recent study, conducted by Mwangi et al., a clinical algorithm containing 17 signs and symptoms was applied to children up to 15 years of age. Among children under five years of age, the algorithm was able to select 84% of those patients with a parasitaemia ≥ 5000 parasites/µl of blood, but had a positive predictive value (PPV) of only 57%. In older children and adults the performance was even poorer (Mwangi et al. 2005).

In 2002 Chandramohan et al. conducted a review of published studies about algorithms for diagnosing malaria. The authors evaluated both, combinations of only

two independent symptoms like fever and chills and/or sweating and score-based algorithms, which consist of up to ten signs and symptoms. Even so, they concluded that the accuracy of clinical algorithms is not sufficient enough to determine, whether antimalarial drugs should be given or withheld to children with febrile illness (Chandramohan et al. 2002).

Algorithms should provide high accuracy for clinical decision-making, but signs and symptoms of uncomplicated malaria overlap with several other conditions. The best-known algorithm for the clinical diagnosis of malaria is the Integrated Management of Childhood Illness (IMCI) - algorithm, which was introduced by the World Health Organization (WHO) in 1997 and which is still the leading guideline worldwide.

1.6 Integrated Management of Childhood Illness

In order to achieve the goals of reducing child mortality in children under five years of age the WHO launched its Integrated Management of Childhood Illness – program in 1997 (Gove 1997). It should be built on the existing health facility structures, which were predominantly minted by minimal or non-existent diagnostic, laboratory and medical resources in primary health care settings. Assessment of children in health facilities in developmental countries did not follow any superior methodology and previous research was not implemented on an evidence basis.

The three main components of the IMCI-strategy are:
- Improving case management skills of health-care staff
- Improving overall health systems
- Improving family and community health practices

Related to the community-level, these aims should result in a more accurate and reliable identification of childhood illnesses through higher qualified personnel and quicker referral of severely ill children to suitable health facilities.

Implementation of appropriate care seeking behavior as well as preventive care in home settings and a more effective use of drugs were additional targets (Gove 1997).

The program was implemented in more than 75 countries worldwide (World Health Organization 2012c) and in 44 of 46 African countries by 2005 (World Health Organization 2012b). To ensure an optimal adaptation to the specific needs and capabilities of each environment, the WHO worked in close cooperation with local governments and ministries of health.

The IMCI - clinical guidelines are designed for use in poor clinical settings and represent one of the main parts of the initiative. The guidelines are described in the "IMCI-handbook" and cover algorithms for assessment of sick children in order to guide the health workers to the correct treatment. Apart from that the guidelines provide structured information about disease prevention, e.g. vaccination or bed net-use as well as nutritional counseling (World Health Organization 2010d).

After individual national adaptations of the handbook's content, it was made available to teaching institutions to incorporate the guidelines in the education of doctors, nurses and other health professionals (World Health Organization 2010c, World Health Organization and Ghana Office 2004).

Ghana started IMCI in 1998 in four pilot districts after initial capacity building. During the year 2000 all districts had started and - according to latest available data - there are currently 33 districts working with IMCI (World Health Organization et al. 2004).

Since the beginning of implementation there is an ongoing, partly well–documented, review process to evaluate efficiency and practicability of IMCI and development of mortality-rates. Several studies showed a major improvement of health workers performance in facilities, where the staff was previously trained in IMCI case management. Children assessed by those health workers received a more appropriate treatment and caretakers were more likely to receive correct instructions about how to continue treatment at home and when to return to the health facility compared to children, who were assessed by "non-IMCI health workers" (Armstrong Schellenberg et al. 2004, El Arifeen et al. 2004).

As implementation of IMCI was adopted on a regional basis one of the consequential postulations was to begin all new child survival efforts with local epidemiology, targeting the major causes of death within each region and even down to the district level (Bryce and Victora 2005).

1.7 Health care setting in Ghana and West Africa

Although Ghana is one of the higher developed countries in West Africa, the health-system is far away from providing sufficient health care to every child. In 2008, 30% of all children under five years of age were stunted and especially the "under-five-survival" in rural regions was still less likely, since 75 children per 1000 live births died in urban regions in 2008 compared to 91 deaths per 1000 live births in rural

areas. The physician density for the entire country of Ghana was only 0.85 per 10.000 people in 2009(World Health Organization 2009).

In contrast to the partially low utilization of health facilities in some other areas, the Ashanti region, where this study took place, is relatively well equipped with overall 530 hospitals and health stations. The majority (53%) is privately managed and almost a third is operated by the governmental Ghana Health Service (Ghana Health Service 2008).

Despite the general deficits, Ghana is also one of the countries that outperformed the average figures of the 'WHO African Region'. For instance, 78% of women received 'antenatal care (4+ visits)' in the year 2008 (vs. an average of 44% of women in the entire WHO African region). For the criterion 'births attended by skilled health personnel' it was 59% (vs. 48%) and for 'measles immunization' it was 93% (vs. 69%) (World Health Organization 2009).

However, there are other reasons existing that keep the overall health situation on a low level: The three main reasons are namely financial barriers, insufficient health care–seeking behavior and traditional beliefs. Financial barriers particularly comprise transport costs to the health facilities, costs for medication and the health service itself since memberships in community-based health insurance schemes (CBHI) are frequently not existing (Boateng et al. 2008, Sarpong et al. 2010, Smith et al. 2008). In Ghana 46.8% of the total expenditure on health had to be paid by the patients (data from 2009) (World Health Organization 2010b). Improvement of health care – seeking behavior, which is also one of the main goals of the IMCI – strategy, has proven to be a complex problem. In 2008 for instance, only 51% of children in Ghana who showed symptoms of an acute respiratory infection were taken to a health facility (World Health Organization 2010b).

Other studies highlighted that mothers purchased drugs for their children without seeking prior medical help (Mbagaya et al. 2005) or presented them to health facilities later than 24 hours after onset of febrile illness in high transmission areas (Deressa et al. 2007). Those inhibitions for seeking medical assistance are often associated with traditional beliefs and trust in traditional healers.The belief of an illness not being treatable by modern medicine may also lead to a lack of recognition of danger signs (Hill et al. 2003). In many countries malaria has different synonyms in each community and is known as a local disease, which is often attributed to evil spirits. Thus, convictions still widely exist that such an illness is only curable by

attending traditional healers (Makundi et al. 2006). Data from 500 interviewed patients with malaria in Ghana showed, that 43% of them had taken anti-malarials prior to hospital attendance: 77% of those had used the drugs inappropriately (Buabeng et al. 2007).

1.8 Aim of the study

The goal of the present study was to generate and evaluate an age-derived clinical algorithm using simple clinical signs and symptoms for the diagnosis of *P. falciparum* parasitaemia. An algorithm such like this could idealley help to detect a focus group of children for presumptive treatment if resources are scarce. To date there is no data on age-derived clinical algorithms available. For better adoption to the clinical decision-making we used a Classification and Regression Tree (CART).

2. Population and Methods

2.1 Study Area

Ghana is located on the Gulf of Guinea in West Africa and consists of a total area of 238.537 km^2 with an estimated population of 20.5 millions. More than 100 different ethnicities live in the country, the Akan (e.g. Ashanti, Fanti, Akim) account for the majority. The present study took place in Agogo, which is located in the Ashanti Akim North District, 80 kilometers east of the regional capital Kumasi (Unicef 2007).

The population in the Agogo District and the neighbored Ashanti Akim North District is estimated with 140.000. Most people generate their income with small agriculture business or as retailers. Cultivation comprises Maize, Cassava, Plantain, Cocoyam and Yam.

Situated in a valley, Agogo is surrounded by secondary forested tropical uplands, which are interspersed with discontinuous farmland. With a fully developed road, Agogo is well connected to the bigger city of Konongo in the southwest. All other villages around Agogo are only reachable via dirtroads, which can be temporarily flooded during the rainy months.

The climate is tropical, with a median-temperature of 27°C and a humidity averaging about 85%. The average yearly precipitation is about 166.7 cm (Ghana Health Service 2008).

Plasmodium falciparum is the predominant malaria parasite species followed by *Plasmodium malariae* in the savanna and *Plasmodium ovale* in rainforest regions and vectors are *Anopheles gambiae* and *Anopheles funestus*(Browne et al. 2000).

Transmission of malaria parasites in the Ashanti region is stable, with an estimated entomological inoculation rate of >400 per year (Kobbe et al. 2007).

In the entire Ashanti region 530 health-facilities were counted. Thirty-two percent of them are operated by the governmental Ghana Healths Service (GHS). With 38%, Kumasi has the highest percentage of facilities (Ghana Health Service 2008).

The Agogo Presbyterian Hospital (APH) is situated at one of the main roads, close to stops of mini busses and taxis. The hospital provides 250 beds in five departments (Surgery, Internal Medicine, Gynaecology and Obstetrics, Pediatrics and Ophthalmology) and employs 19 physicians. Besides, the hospital maintains outstanding certified laboratory facilities (Agogo Prebyterian Hospital 2010). The pediatric department is divided into the Child Welfare Clinic (Outpatient Department),

a neonatal intensive care unit (nursery) and the children's ward as well as a maternity.

The Outpatient department offers health care to children from birth up to 15 years of age and is equipped with simple devices such as weighing scales, stadiometer, thermometers, consulting tables and two examination couches. It is operated by a team of two pediatricians and five nurses as well as two health workers.

2.2 Data collection

2.2.1 Study period

All children aged up to 15 years, who were presented to the outpatient department by their parents or caretakers in the period between May 2007 and July 2009 were included in the study.

2.2.2 Inclusion and exclusion criteria

All children in the age of 15 years or below who were attending the OPD for any kind of illness were initially eligible for inclusion in the study. The legal guardian of the child had to sign the informed consent after in-depth explanation of the purpose of the study.

In case of withdrawal of the legal guardian to sign the informed consent or to provide blood samples for examination, the patient was excluded from the study population.

2.2.3 Recruitment of patients in the Outpatient department

In the present study individuals could be included for multiple times. Upon registration in the OPD the study nurse had to decide whether the individual had to be defined as a new patient or a review. For the analysis of this study a "patient" was defined as an individual visiting the OPD. To be classified as a new patient (i.e. "an individual with a new episode of illness") the individual had to attend the OPD without any prior visit within the last 14 days. Individuals who had already taken part in the study and who attended the OPD for the second time within 14 days were handled as a review and were not included in further analyses. Below, the term 'patient' will be used for the single visit of one individual according to this definition.

Each patient below 15 years of age who presented to the OPD within the study period, was registered in the ward book by a study nurse on duty at the registration counter. Afterwards, body temperature, weight and height were measured and

vaccine immunization state was documented. After a member of the study team has ensured, that the patient was not already registered as a participant of the study, the parents or guardians were asked for consent of enrolling the child in the study. If they agreed they had to sign the informed consent form (ICF). If the parents or legal guardians of the child were illiterate, they were asked to provide a thumbprint. ICFs of parents or legal guardians who were illiterate had to be signed by a witness too.

Afterwards a case report form (CRF) and a lab sheet were prepared and the patients were forwarded to an experienced pediatrician, who conducted a detailed interview with the child and/or parents and performed a physical examination. All findings and the diagnosis were entered in the CRF.

Each patient was asked to provide an EDTA-blood sample for a blood count and a sample for a malaria slide. After both of these results were available, the patient returned to the pediatrician, who decided, whether the patient had to be referred to the ward or could be sent home after treatment.

2.2.4 Case Report Form (CRF)

The case report form (CRF) is a three page–document that had to be filled out for each patient.

The personal data on the front page, as well as state of vaccine immunization, weight, height and body temperature on admission, were entered by the study nurse on duty at the registration counter.

The CRF was designed in two rows, of which one had to be filled out by the admitting study nurse at the registration and the second had to be completed by a doctor.

Where the nurse mostly had to choose between binary options (e.g. "yes" or "no", "productive cough" or "dry cough"), the doctor had more space for individual comments and qualitative assessment. Since laboratory results were only available after a while, all signs and symptoms (e.g. *palmar pallor*) were assessed subjectively. On the second half of the last page the pediatrician had to state a final diagnosis, which should be confirmed by laboratory findings. Further it was necessary to note whether the patient had to be admitted to the ward and which prescriptions have been made.

2.2.5 Laboratory Request Form

The laboratory request form was also prepared for each patient, who was enrolled in the study. The doctor who assessed the child in the outpatient department had to choose, which tests are needed to confirm the suspected diagnosis. Together with the blood samples the laboratory request form was then forwarded to the laboratory, where all results of the analyses were written down on the form. Except of special analyses for clinical chemistry, which were sent to the Komfo Anokye Teaching Hospital Kumasi, all common examinations were performed in the hospital's laboratory and results were at latest available within a few hours.

2.2.6 Data Management

For each individual a study folder was created, which contained all CRFs, laboratory request forms and other patient related documents. The folder remained in the OPD, until it was completed and a diagnosis was made. Afterwards, the folder was forwarded to the data entry clerks, who double-entered the data within 48 hours. For data entry, the software "4th Dimension" was used. Inconsistencies and needs of clarifications were managed by the data manager, who was also responsible for cross–checking the data. Once in a month data synchronization with the Bernhard-Nocht-Institute of Tropical Medicine (BNITM) in Hamburg was performed. A list with inconsistencies was sent back to Agogo to be corrected in the database by the data entry clerks. At all other times the folders were stored in a locked room with access for study personnel only.

2.2.7 Consent and Ethical Approval

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki, and consistent with Good Clinical Practice (GCP) and the applicable regulatory requirements. Ethical approval for the study was obtained from the respective responsible Ethics committees, namely from the School of Medical Science, Kwame Nkrumah University of Science and Technology(KNUST), Kumasi. Mothers of children were informed about the aim of the study in presence of a witness and their understanding was assessed by a set of standard questions Informed consent was sought from mothers who granted it by signature or thumb print.

2.2.8 Data Management and Analysis Software

Data Entry and Evaluation was performed using 4th Dimension Data Management software (4D SAS, France). Data analyses were carried out by using STATA IC/10.0 Statistical and Data Analysis Software (Stata Cooperation, California).

2.3 Methodology of laboratory examinations

2.3.1 Blood count

For analysis of the capillary blood samples, a Sysmex® KX-21N Haematological Analyzer (Sysmex Corporation, Kobe, Japan) was used. The machine was tested and calibrated on a daily basis. A three level control (Eightcheck-3WP controls (Low, Normal and High)) was run each morning to ensure the function of the machine.

The counter was able to provide a full blood count as well as differentiation of white blood cells within a few minutes.

2.3.2 Malaria slide

For detection of malaria parasites, a thin and a thick blood film were prepared for each child enrolled in the study. Therefore capillary blood was collected out of the fingertip after alcoholic skin disinfection. To avoid dilution, the first three drops of blood were discarded.

For preparation of the blood slide a single drop of blood was placed on a object slide and was then greased out with the help of a second object slide, held in an angle of 30° to 45°. For the thick blood film three drops of blood were collected on the object slide and mingled instantly to form a thick layer of blood.

Both slides were fixed in pure methyl alcohol, after they were dried. After renewed drying, the slides were stained in Giemsa Solution (10%) with a pH between 7.0 and 7.2 for 10 to 15 minutes. Afterwards they were rinsed with clear water and put in a vertical position for drying.

For counting the parasites, the lab technician used Immersion oil microscopy with 100x magnification. After seeking for a visual field showing eight leukocytes in the thick blood film, parasites were counted simultaneously to the leucocytes, until 200 leucocytes were counted. According to the current blood count, parasites were calculated up to 1 µl of blood.

If there was no blood count available, an average of 8000 leucocytes per µl of blood was estimated (Greenwood and Armstrong 1991). If there were no parasites visible

after counting of leucocytes, the slide was decided to be negative. In cases of very high parasite densities, the parasite count was performed with the thin blood film, which was made out of a single drop and parasite densities were counted up to 1000 erythrocytes.

2.4 Study population and processing of database for analysis

2.4.1 Preparation of datasets for analysis

During the study period between May 2007 and July 2009, 4981 individuals between two months and five years of age were included in the study population. As many of them visited the OPD twice and more, a total of 8283 patients were registered in the database (Figure1).

```
┌─────────────────┐     ┌──────────────────────────────────────┐
│   Enrollment    │     │ Assessed patients[a] (n=8283)        │
└─────────────────┘     │                                      │
                        │          - 4981 individuals          │
                        └──────────────────────────────────────┘
                                          │
                                          │      ┌──────────────────────────────────┐
                                          │      │ Excluded  (n= 2836)              │
                                          └─────▶│ ♦ Patients with missing CRF –    │
                                                 │   information[b] / missing malaria│
                                                 │   smear result                   │
                                                 └──────────────────────────────────┘

┌─────────────────┐     ┌──────────────────────────────────────┐
│    Analysis     │     │ Eligible patients[a] (n=5447)        │
└─────────────────┘     │                                      │
                        │          - 3641 individuals          │
                        └──────────────────────────────────────┘

        ┌──────────────────┐          ┌──────────────────┐
        │   Patients[a]    │          │   Patients[a]    │
        │  2 – 12 months   │          │  12 – 60 months  │
        │   (n= 1304)      │          │   (n= 4143)      │
        └──────────────────┘          └──────────────────┘
```
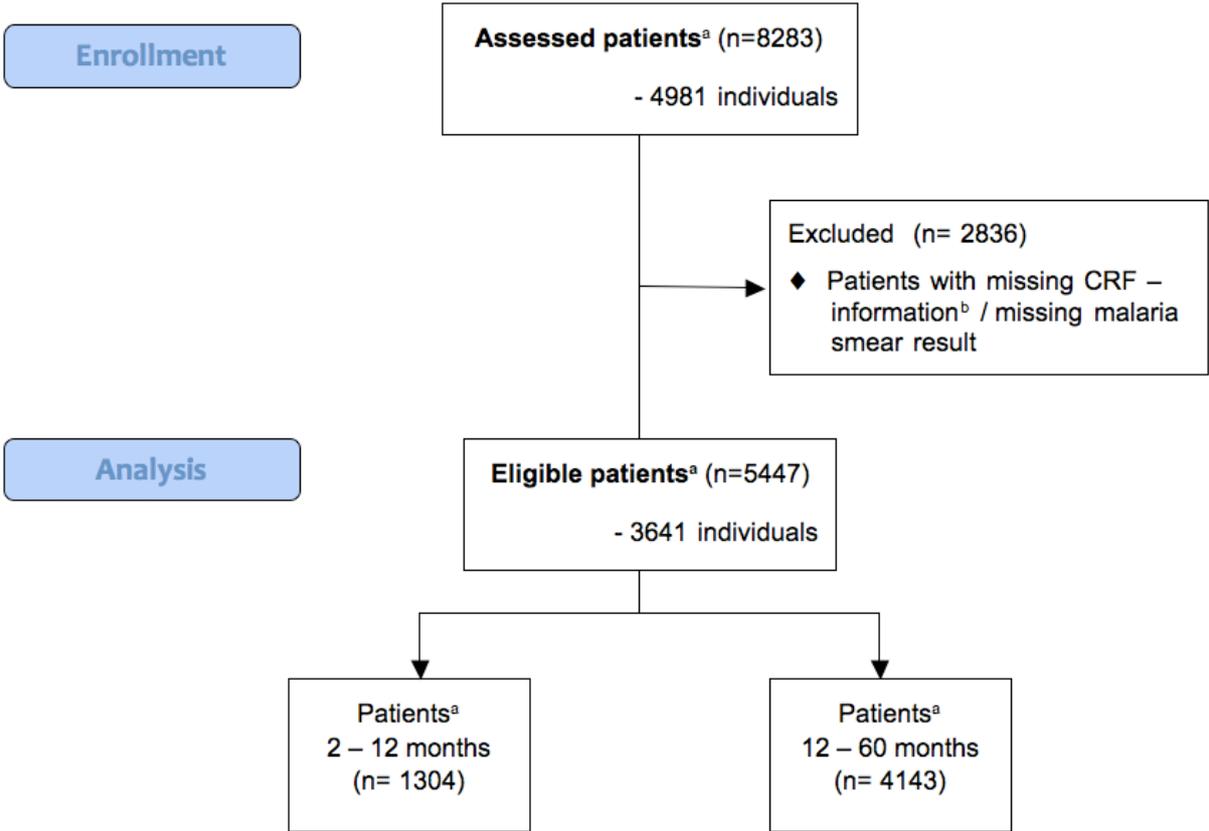
Figure 1. Enrollment and exclusion of patients aged 2 months to five years

[a] A patient was defined as an individual visiting the OPD. [b] Case report forms must have information for each variable in Table 1 available.

All visits of children who were younger than two months or older than sixty months at the time of their visit were not included in the analysis. Consequently, it was possible that one individual had three visits during the study period, but was included only once because of exceeding the age limit of five years after his/her first visit.

A set of variables was compiled which had to be available for each patient to ensure comparability and consistency. Patients who had missing values for one of the variables in Table 1 were excluded from the analysis. Patients for who no malaria count – result was available were also excluded.

After exclusion of all patients with incomplete datasets, 5447 patients (3641 individuals) were left (Figure 1).

2.4.2 Preparation of variables

Initially, values for more than 150 different variables were transferred from the CRF into the database. First analyses revealed that for several of these variables no values were available. To ensure high consistency throughout the dataset it was necessary to consider only those variables that have been assessed in all patients. Therefore it was evaluated which variables could be excluded without reducing the total number of patients in the analysis by more than 10%.

This attempt should prevent loosing disproportional numbers of cases, only to have a single variable available for each case that is potentially not important for the analysis.

Some of the variables were direct translations of questions (e.g. "cough: yes/no"), others were completely new generated out of text-statements (e.g. "child is poorly feeding"). Finally all variables used for analysis were binary information.

2.4.3 Data analysis

After description of the distribution of sex, age, personal data, parasitaemia and signs and symptoms, each variable from Table 1 was put in a bivariate regression analysis to compute Odds Ratios between symptoms and *P. falciparum* parasitaemia (Table 2). The resulting Odds Ratios were used to decide whether the variables were handled as potentially predictive.

Table 1. Variables for which values were required for inclusion

Variable[a]	Short definition
Nutrition changed to poor feeding	Reduced eating or breastfeeding during the last 5 days
Convulsions	Report of convulsions
Elevated body temperature	Measured axillary body temperature of 37.5°C or above
Report of fever	History of fever within the last five days
Prostration	Abrupt failure of function or complete physical exhaustion
Cardiac fatigue	Any signs of cardiac fatique (e.g. pretibial oedema or dyspnoea)
Cyanosis	Any signs of cyanosis (e.g. on the lips or nailbeds)
Skin rash	Any visible rash on the skin
Skin depigmentation	Any visible depigmentation of the skin
Skin abnormalities / Other skin problem	Any skin abnormalities that are not rash or depigmentation
Palmar pallor	Pallor of the palm (e.g. in comparison to the hand of the physician)
Jaundice	Any visible jaundice of the sclera or the skin
Cough	History or presence of cough
Breathing difficulties	Stressed and/or impaired breathing, inability to breath deeply
Breathing fast	Increased breathing frequency (2-12 months of age: ≥50 breaths/min, 12 months – 15 years: ≥40 breaths/min)
Deep breathing	Increased deepness of breathing (e.g. as compensation of metabolic acidosis)
Respiratory distress	Severe respiratory impairment
Chestindrawing	Visible indrawing of the chest wall in inspiration (syn. subcostal indrawing)
Running nose	History or presence of running nose
Blocked nose	History or presence of blocked nose
Diarrhoea	Loose stools with a frequency
Vomiting	History or presence of vomiting
Drinking thirsty	Increased sensation of thirst and increased quantity of drunken fluid
Pinch abdomen present	Reduced skin turgor (e.g. in case of dehydration)
Earpain	History or presence of earpain
Malnourishment	Subjective impression of malnourishment

[a]Variables were binary coded (0;1), patients included in the analyses must have a value avaible for each variable in this table

Those variables providing both, an Odds Ratio ≤0.83 or ≥1.20 and an occurrence in at least 1% of cases with present parasitaemia were put in a forward and a backward stepwise logistic regression. For variables with an Odds Ratio of ≤0.83 bunched inverse variables were generated (e.g. *no respiratory symptoms*). To be positive for this inverse variable a patient must not present a symptom of the respective group of symptoms (e.g. for *no respiratory symptoms*: no *chestindrawing,* no *breathing difficulties,* no *fast breathing,* no *running nose,* no *blocked nose* and no *cough*).

2.4.4 Stepwise logistic regression

Variables, which met the criteria regarding a potential predictive value, were first entered into a forward stepwise logistic regression to determine the minimum number of independent variables necessary to accurately estimate the outcome.

The outcome was defined as *Plasmodium falciparum* parasitaemia of any density, detected by either thin or thick blood film.

The model selected the variable strongest related to the outcome and entered it into the model first. Following variables were selected according to the best improvement to the fit of the model after adjustment to all other variables.

At a certain point, where none of the remaining independent variables improved the fit of the model significantly, entering of new variables was stopped. For statistical discrimination a p-value of 0.05 was set as cutoff.

Backward stepwise regression proceeded inversely and entered all independent variables first. Due to the better ability of backward logistic regression models in determining suppresser effects and the need of testing the robustness of the model, it was also performed with all independent variables (Lemeshow S and Hosmer DW jr. 2000).

Variables that were estimated as being predictive by both parameter reduction methods were then entered in the CART – analysis.

2.4.5 Classification and regression tree analysis (CART)

CART-Analysis, also known as '*Recursive Partioning'*, is a technique for separating the independent variables, i.e. the occurrence of a certain sign or symptom, into subgroups based on the outcome.

Using the Martingale Residuals of a Cox regression model to calculate approximate chi-square values for all possible cut-points on all of the CART covariates, this method results in calculation of individual relative hazard rates.

Beginning with a parent node containing all cases of a specific age group, CART is continuously trying to maximize the purity, i.e. to segregate children who are affected by parasitaemia from those children who are not affected. Therefore, the model generates a decision tree by splitting the cases of the parent node at an optimal split point where the arising child nodes provide greater purity (better segregation of affected and non-affected children). For detection of the optimal split point the entered binary prediction variables were used.

Every child node in turn can become a parent node, as CART continues the splitting process until statistical analysis indicates that another child node would not be of greater purity (Marshall 2000, Speybroeck 2011). The resulting tree is structured as a sequence of yes/no questions. For calculation of cut-points a p-value of <0.05 and a minimum size of the subgroup of 10 cases were set.

The target dimension of a Cox regression is the time until occurrence of a certain event, i.e. death or illness. The effect of the analyzed explanatory variable during this time is described as Hazard Ratio. CART therefore requires the input of a certain time variable. In this study all enrolled children had already reached the stage of experiencing the outcome, in the sense of being positive or negative for parasitaemia. As all signs or symptoms, which were described by the pediatrician, were assessed at the same time and no follow-up assessment was conducted, time was treated as a dummy variable, being '1' for any assessed symptom.

By using this attempt, the influence of the time variable was disconnected from the CART model. CART then simply worked as a method of describing the strength of association or non-independence between two binary data values, by calculation of the expected number of events for each subject within a subgroup.

Concerning this variation, the regular output of a Hazard Ratio can be regarded as Odds Ratio as well (Speybroeck 2011).

CART - analysis was performed separately for children aging 2 – 12 months and for children between 12 – 60 months of age.

2.4.6 Classification of models

Sensitivity, specificity, negative (NPV) and positive predictive values (PPV) were calculated for each CART model and for the Integrated Management of Childhood Illness – algorithm. Single signs and symptoms, as well as combinations of those, were also classified.

2.4.7 Testing null hypotheses

For testing the null hypothesis on the significance of different haemoglobin (Hb) concentrations in patients with *palmar pallor* an unpaired two-sample t-test was performed, assuming a Student's t distribution.

2.5 Clinical definitions

2.5.1 Report of fever

A *report of fever* was defined as any history of fever or 'feeling hot' within five days prior to the visit reported by the legal guardian of the child. Because households in the Ashanti region lack in thermometers, most of the febrile episodes could not be confirmed with thermometers at home.

2.5.2 Elevated body temperature

An *elevated body temperature* was defined as a body temperature of 37.5° Celsius or above (≥ 37.5°C). The temperature elevation was always measured with a thermometer by one of the study nurses upon assessment of the patient.

2.5.3 IMCI definitions

The World Health Organization (WHO) recommends classification of febrile children based on the malaria risk of the area. In *high malaria risk* areas like Ghana where more than 5% of fever cases are due to malaria, two IMCI classifications are applicable:

-*Very severe febrile disease*
Children who suffer from a very severe febrile disease according to the WHO guidelines, have to show any of the general danger signs (unability to drink or breastfeed, vomiting everything, convulsions, unconsciousness or lethargy) or a stiff neck.

- *Malaria*

If a child has a fever (by history, feeling hot, or a temperature of 37.5°C and above), but no general danger sign, then a child is classified as a case of malaria. Parasitological laboratory diagnostics are not required for this definition.

2.5.4 Age groups

For the CART analysis the study population was stratified in two age groups.

- Age group 1: Children between ≥2 and <12 months of age
- Age group 2: Children between ≥12 and <60 months of age

Instead of writing "between ≥2 and <12 months" or "≥36 - <48 months" in some figures and tables it was just written "between 2 and 12 months" or "36 – 48 months" for better readability.

3. Results

3.1 Describing the population

During the study period 5447 patients were enrolled for whom all required data for analysis was available. Beginning in May 2007 the number of monthly study recruitments rose up to a maximum of 450 in the month of April 2008. During the last 8 months of recruitment until July 2009 the OPD was not well staffed with personnel and only smaller numbers of cases were registered. Apart from this no significant seasonal variations could be detected (Figure 2).
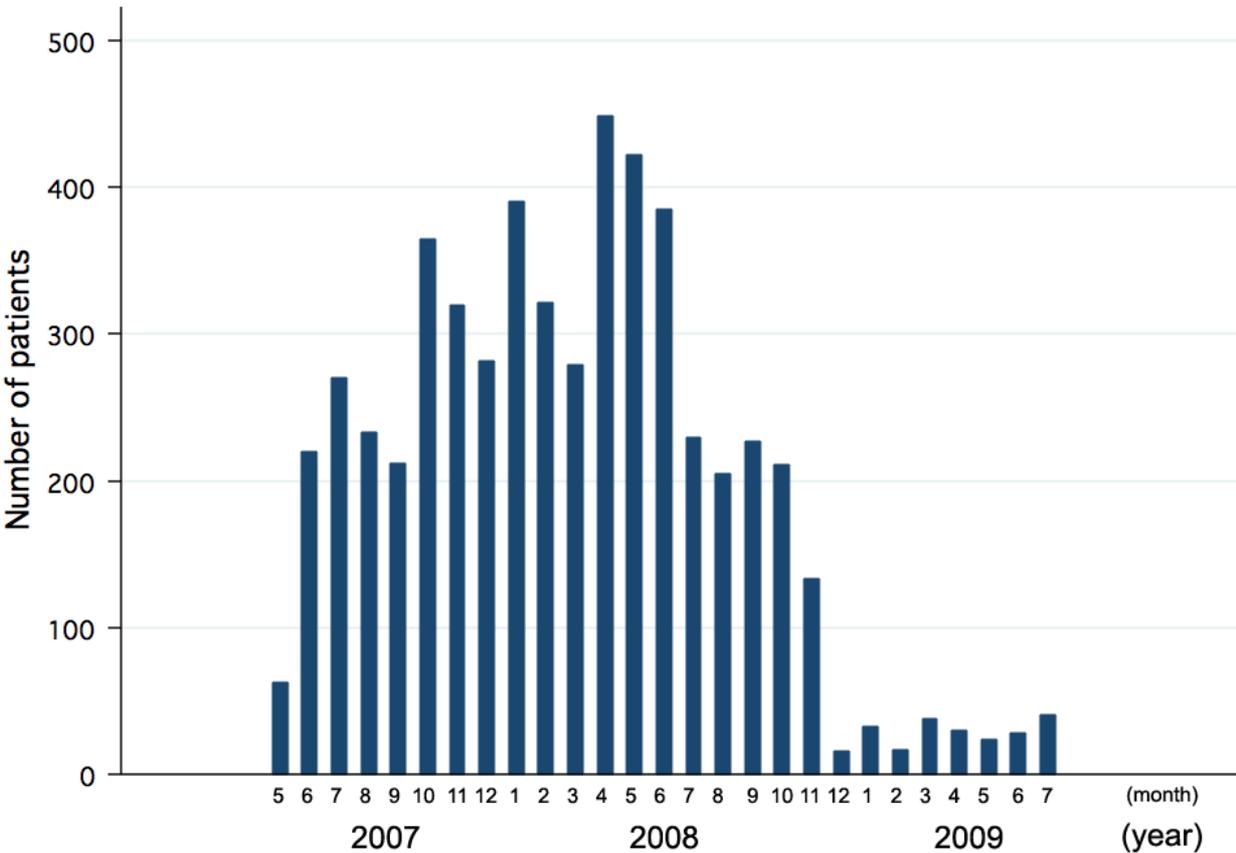
Figure 2. Enrollment of 5447 patients during the study period between May 2007 and July 2009

For the present study a patient was defined as an individual visiting the OPD, whereby it was possible for an individual to be included for multiple times according to the inclusion criteria. Thus single and multiple visits of 3641 individuals result in a total of 5447 patients in the analyses. As Figure 3 demonstrates 2516 (69.1%) of all registered individuals visited the OPD only once. Six-hundred-ninety-seven (19.1%)

children presented to the clinic twice and 261 (7.2%) patients came for three times (Figure3).
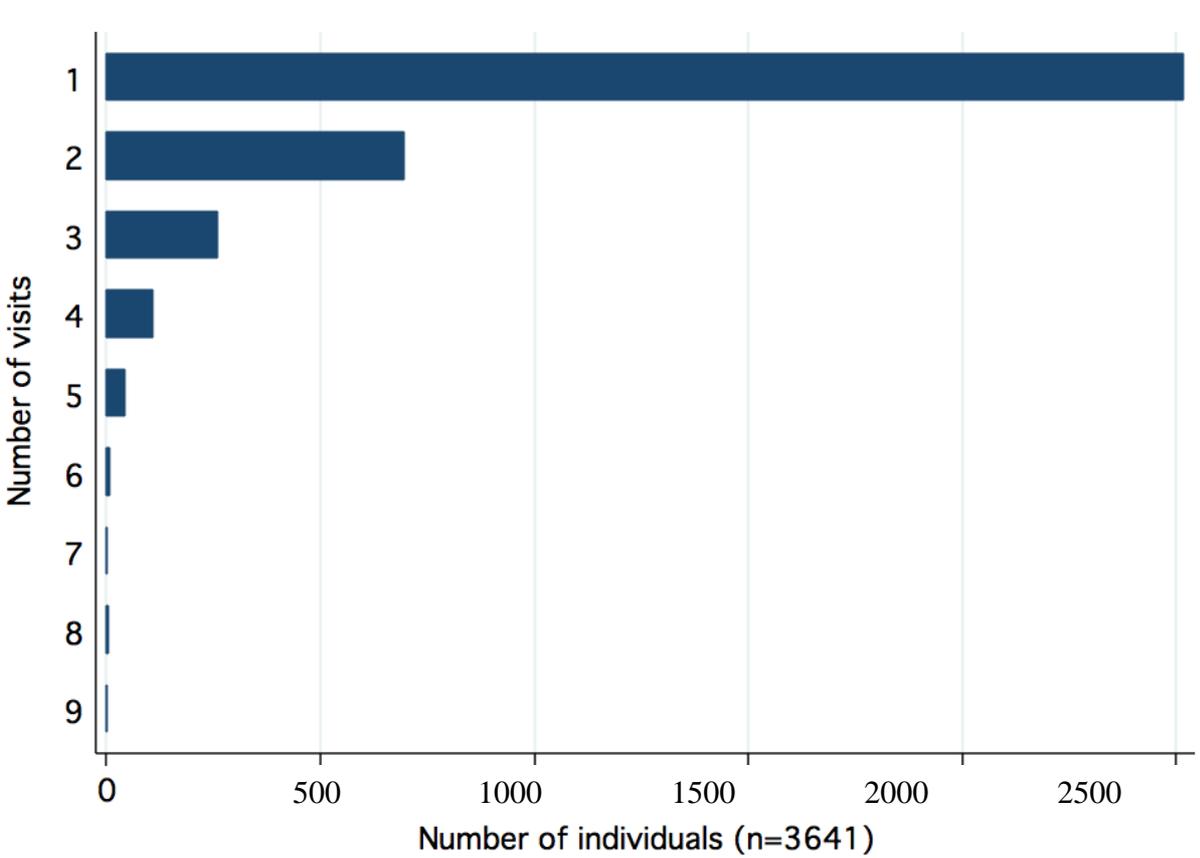
Figure 3. Distribution of single and multiple visits of 3641 individuals

Table 2 shows that sex was almost equally distributed among the registered children with 2882 (52.9%) male patients and 2565 (47.1%) female patients. Grouped into two age groups (2-12 months and 12-60 months), the fraction of children aged between 2 and 12 months was smaller with 1304 enrolled patients compared to 4143 patients between 12 and 60 months, respectively.

According to their parents or guardians the majority of children belonged to the ethnic group of Akan (84.5% of patients 2-12 months of age and 82.6% of patients 12-60 months of age). This group was followed by the ethnicities Northeners (10.4% vs. 12.3%), Ewe (2.1% vs. 2.4%), Ga (0.8% vs. 0.9%) and Others (2.2% vs. 1.8%).

Regarding patients in all ages over 53% (2921) of the patients came from outside of Agogo to present to the Outpatient department, 46.4% (2526) resided in Agogo itself.

Table 2. Distribution of sex, age, ethnicity and origin among the study population

	Patients 2-12 months (n=1304)	Patients 12-60 months* (n=4143)	Total (n=5447)
Sex			
Male	713 (54.7%)	2169 (52.4%)	2882 (52.9%)
Female	591 (45.3%)	1974 (47.7%)	2565 (47.1%)
Ethnicity			
Akan	1102 (84.5%)	3420 (82.6%)	4522 (83.0%)
Ewe	27 (2.1%)	98 (2.4%)	125 (2.3%)
Ga	11 (0.8%)	39 (0.9%)	50 (0.9%)
Northeners	136 (10.4%)	510 (12.3%)	646 (11.9%)
Others	28 (2.2%)	75 (1.8%)	104 (1.9%)
Residence			
Agogo	524 (40.2%)	2002 (48.3%)	2526 (46.4%)
Outside Agogo	780 (59.8%)	2141 (51.7%)	2921 (53.6%)

* One patient had missing information for "ethnicity"

3.2 *P. falciparum* parasitaemia, fever and 'clinical malaria'

Disregarding the clinical presentation or any suspected diagnosis every patient was asked to provide a blood sample for preparation of a thin or thick blood film.

The prevalence of *P. falciparum* parasitaemia was 13.8% in children aged 2-12 months and 30.6% in children between 12 and 60 months of age, which resulted in an overall prevalence of parasitaemia of 26.6% regarding the full age range (Table 3).

In Figure 4 and Figure 5, the distributions of *P. falciparum* parasitaemia and 'clinical malaria' are visualized. Both of the prevalences were increasing up to the age of 36 months, with a peak prevalence of 38.5% for *P. falciparum* parasitaemia and 36.0% for 'clinical malaria', respectively for the children aged between three and four years. The youngest children aged between 2 and 12 months were least affected with prevalences of 13.8% for parasitaemia and 12.1% for 'clinical malaria' respectively. As almost all children with a parasitaemia, had an *elevated body temperature* or a *report of fever*, they met the case definition of having malaria.
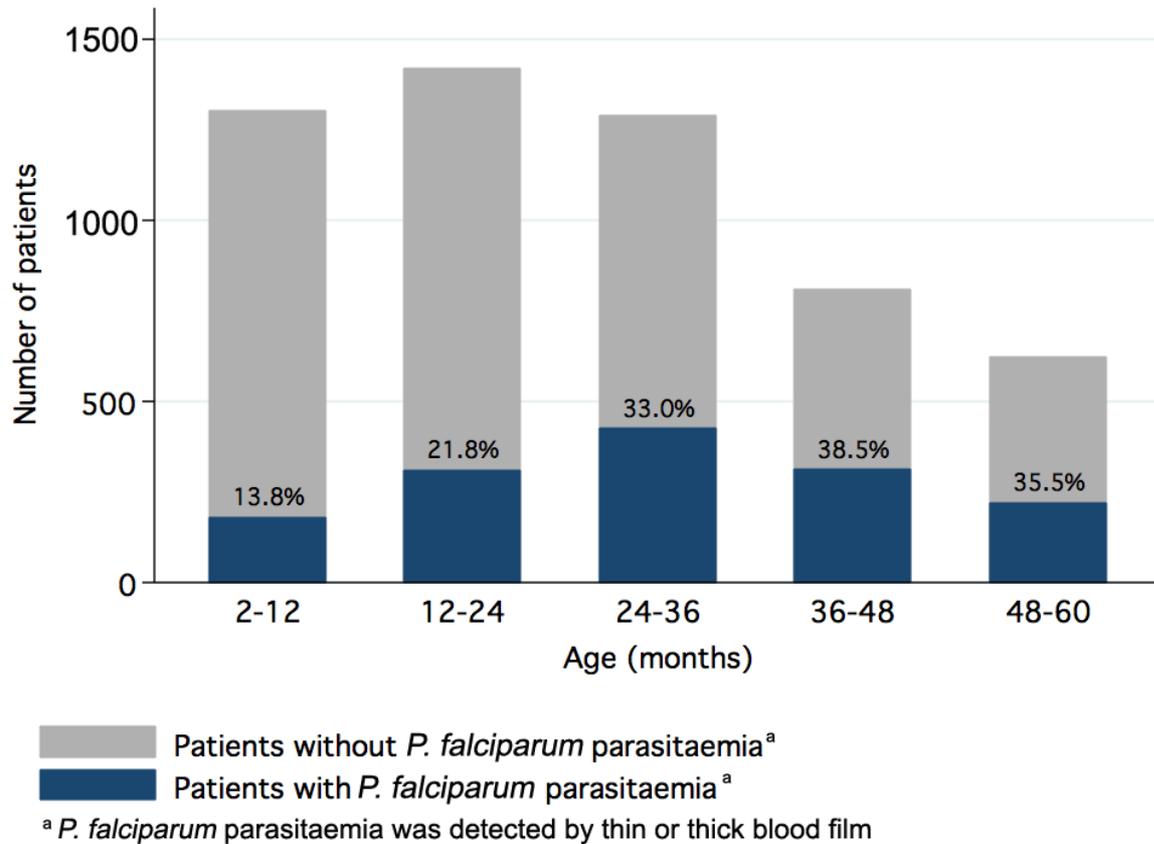
Figure 4. Age distribution of P. falciparum parasitaemia in 5447 patients

Body temperature was measured for each child enrolled in the study and the legal guardian was additionally asked for presence of fever within the last five days.

Irrespective of the age, the percentage of children with an *elevated body temperature* on admission was significantly higher with 882 (60.9%) patients in the group of patients with detected parasitaemia, compared to 1134 (28.6%) children with a body temperature equaling to 37.5°C or above and a negative malaria film (Table 4).

If only a report of fever was considered as a criterion for having fever, in general more of the admitted patients were found to have experienced an episode of fever with a total number of 1401 (96.7%) patients with positive malaria films and 3167 (79.2%) parasite negative patients, respectively.
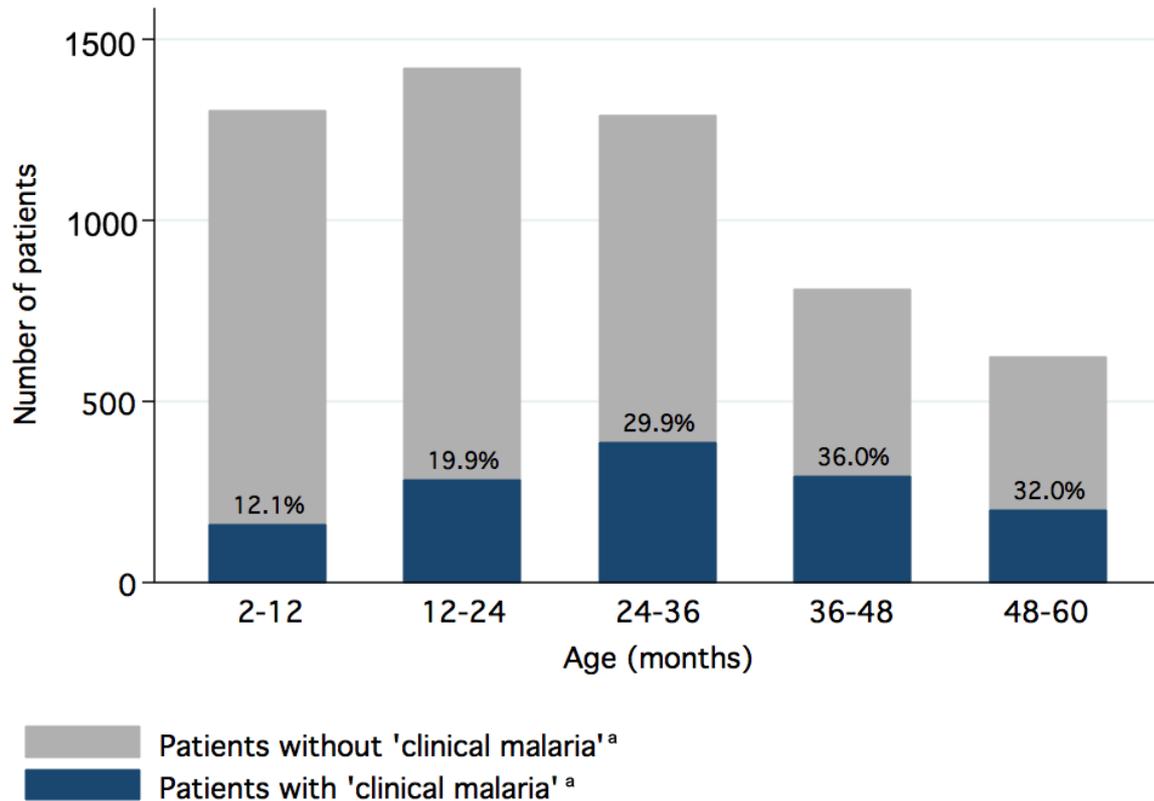
Figure 5. Distribution of diagnosis 'clinical malaria' in patients of different age

The vast majority of children (83.0%) has received one or more doses of anti-pyretics (e.g. Paracetamol) before attending the outpatient department. Nevertheless, most of the admitted children with a positive blood film had an elevated body temperature. Among children with a negative malaria slide, only 1143 (28.6%) had a persisting elevated temperature. Comparing the different age groups among children with positive parasite state, it is obvious that the role of fever increases slightly with the age, no matter which definition of fever is used (Table 4).

Table 3: Prevalence of *P. falciparum* parasitaemia

Parasitaemia[a]					
Patients 2-12 months (n=1304)		Patients 12-60 months (n=4143)		All patients (n=5447)	
No	Yes	No	Yes	No	Yes
1124 (86.2%)	180 (13.8%)	2874 (69.4%)	1269 (30.6%)	3998 (73.4%)	1449 (26.6%)

[a] *P. falciparum* parasitaemia of any density was detected by thin or thick blood film

Table 4: Application of different definitions of fever on patients with and without *P. falciparum* parastiaemia

	Patients 2-12 months		Patients 12-60 months		All patients	
	Parasitaemia[a]		Parasitaemia[a]		Parasitaemia[a]	
	No (n=1124)	Yes (n=180)	No (n=2874)	Yes (n=1269)	No (n=3998)	Yes (n=1449)
Elevated body temperature ($\geq 37.5°$)	354 (31.5%)	102 (56.7%)	789 (27.5%)	780 (61.5%)	1143 (28.6%)	882 (60.9%)
Report of fever [b]	857 (76.3%)	172 (95.6%)	2310 (80.4%)	1229 (96.8%)	3167 (79.2%)	1401 (96.7%)
Paracetamol taken	782 (69.6%)	162 (90.0%)	2004 (69.7%)	1040 (82.0%)	2786 (69.7%)	1202 (83.0%)

[a] *P. falciparum* parasitaemia of any density was detected by thin or thick blood film
[b] A report of fever was defined as any history of fever within the last five days

3.3 Distribution of clinical diagnoses and signs and symptoms

After completing the examination of each child, the pediatrician had to state a clinical diagnosis. In Table 5 all clinical diagnoses are sorted by frequency for each age group. In both groups "malaria" accounts for the first or second most frequent diagnosis, as 2264 (54.6%) children aged 12-60 months and 494 (37.9%) children aged 2-12 months were diagnosed with malaria. Regarding the whole study population 50.6% (2758) of patients were diagnosed with malaria.

The second most common diagnosis (beyond the youngest children it was even the most frequent) was an upper respiratory tract infection ("URTI").

 "Otitis media" and "Gastroenteritis" are two other diagnoses that rank beyond the "top five" diagnoses in both age groups. "Otitis media" was almost equally distributed in children 12-60 months of age (11.9%) and in children between 2 and 12 months of age (11.4%). "Gastroenteritis" was diagnosed in every fifth child (19.3%) of the youngest children (2-12 months of age) and in 11.6% of children between 12 and 60 months of age. "Anaemia" was diagnosed in different graduations of severity, commonly according to the clinical presentation of the patient.

In both age groups "moderate anaemia" was the most frequent form of anaemia with 2.6% in children 2-12 months of age and 3.4% in children aged 12-60 months.

For each patient a detailed case report form with all clinical signs and symptoms was completed. In Table 6.1 and 6.2, all signs and symptoms (variables) are listed in three categories. The first category lists the variables, which were assessed by the physician or nurse, the second gives information about variables, which were taken from the history of the child. The third category informs about variables, which were generated during the analyses.

Sorted by frequency and irrespective of the generated variables, the most common symptoms in patients without parasitaemia are a *report of fever* in 2647 (66.2% of all patients without parasites) children, *nutrition change to poor feeding* in 2617 (65.5%) patients, *cough* in 2500 (62.5%) patients, *running nose* in 2466 (61.7%) children and *vomiting* in 1275 (31.9%) children.

Table 5. Distribution of clinical diagnoses

Diagnosis*	Patients 2–12 months n=1304 (%)	Diagnosis*	Patients 12-60 months n=4143 (%)	Diagnosis*	Patients 2-60 months n=5447 (%)
URTI [a]	604 (46.3)	Malaria	2264 (54.6)	Malaria	2758 (50.6)
Malaria	494 (37.9)	URTI [a]	1642 (39.6)	URTI [a]	2246 (41.2)
Gastroenteritis	252 (19.3)	Otitis media	491 (11.9)	Gastroenteritis	731 (13.4)
Otitis media	149 (11.4)	Gastroenteritis	479 (11.6)	Otitis media	640 (11.7)
Bronchopneumonia	88 (6.7)	RTI [c]	172 (4.2)	Bronchopneumonia	245 (4.5)
UTI [b]	54 (4.1)	Bronchopneumonia	157 (3.8)	RTI [c]	224 (4.1)
RTI [c]	52 (4.0)	Moderate Anaemia	139 (3.4)	UTI [b]	185 (3.4)
Malnutrition	36 (2.8)	UTI [b]	131 (3.2)	Moderate Anemia	174 (3.2)
Moderate Anemia	34 (2.6)	Malnutrition	90 (2.2)	Malnutrition	126 (2.3)
Impetigo	30 (2.3)	Tinea capitis	75 (1.8)	Enteritis	79 (1.5)
Enteritis	24 (1.8)	Tonsillitis	58 (1.4)	Tinea capitis	76 (1.4)
Severe anemia	20 (1.5)	Enteritis	55 (1.3)	Severe anemia	73 (1.3)
Dysentery	16 (1.2)	Severe Anaemia	53 (1.3)	Dysentery	69 (1.3)
Conjunctivitis	14 (1.1)	Dysentery	53 (1.3)	Tonsillitis	58 (1.1)
Sepsis	14 (1.1)	Helminthiasis	47 (1.1)	Conjunctivitis	53 (1.0)
Pneumonia	9 (0.7)	Anaemia	40 (1.0)	Helminthiasis	49 (0.9)
Giardiasis	8 (0.6)	Conjunctivitis	39 (0.9)	Impetigo	48 (0.9)
Anemia	7 (0.5)	Pneumonia	36 (0.9)	Anemia	47 (0.9)
Septic skin lesion	4 (0.3)	Septic skin lesion	36 (0.9)	Pneumonia	45 (0.8)
Lobarpneumonia	3 (0.2)	Sepsis	30 (0.7)	Sepsis	44 (0.8)
Helminthiasis	2 (0.2)	Lobarpneumonia	28 (0.7)	Septic skin lesion	40 (0.7)
Tinea capitis	1 (0.1)	Giardiasis	23 (0.6)	Giardiasis	31 (0.6)
Tonsillitis	0 (0.0)	Impetigo	18 (0.4)	Lobarpneumonia	31 (0.6)
Enteric fever	0 (0.0)	Enteric fever	12 (0.3)	Enteric fever	12 (0.2)

* Patients could have multiple diagnoses
[a]URTI = Upper Respiratory Tract Infection, [b]UTI = Urinary Tract Infection, [c]RTI = Respiratory Tract Infection

For those children who had parasites in their blood, the most predominant symptoms were a *report of fever* in 1212 patients (83.6% of all children with parasites), *nutrition change to poor feeding* in 1042 (71.9%), an *elevated body temperature* in 882 (60.9%) children, *running nose* in 608 (42.0%) children and *vomiting* in 595 (41.1%) of the children.

Considering a difference in the frequency of presenting the variable of at least five percent, some variables were more often presented in patients with parasitaemia than in those patients without parasites: *Palmar pallor* (17.9 % in parasitic patients versus 5.5% in non – parasitic patients), *report of fever* (83.6 % vs. 66.2%), *nutrition change to poor feeding* (71.9% vs. 65.5%) and *vomiting* (41.1 % vs. 31.9%).

Patients without detected parasites in their blood more often showed a *running nose* (61.7% in non-parasitic patients versus 42.0% in parasitic patients), *skin rash* (13.3% vs. 6.3%), *cough* (62.5% vs. 40.1%) and *diarrhoea* (31.6% vs. 25.7%). Signs of severe illness like *prostration*, *cyanosis*, *convulsions* and *respiratory distress* were not frequently assessed in the OPD.

Each variable from Table 6.1-2 was put in a bivariate regression analysis to compute Odds Ratios between symptoms and *P. falciparum* parasitaemia. The resulting Odds Ratios were used to decide whether the variables were handled as 'potentially predictive'. For variables with an Odds Ratio ≤0.83 bunched inverse variables were generated (e.g. *no respiratory symptoms*). These bunched variables exclude all variables of a group of symptoms (e.g. all respiratory symptoms) at the same time.

Regarding patients with parasitaemia, 637 (44.0%) showed no respiratory symptoms (i.e. were negative for *respiratory distress*, *chestindrawing*, *breathing difficulties*, *fast breathing, deep breathing, running nose, blocked nose and cough)* at the time of assessment. Among patients without detected parasites this proportion of patients with absence of respiratory symptoms was much smaller with 1002 (25.1%) patients. The resulting Odds Ratios were used to decide whether the variables were handled as 'potentially predictive' for *Plasmodium falciparum* parasitaemia. Those variables providing both, an Odds Ratio of ≥1.20 and an occurrence in at least 1% of cases with present parasitaemia were put in a backward and forward stepwise logistic regression.

Table 6.1. Signs and symptoms and their association with *P. falciparum* parasitaemia in children

Signs and Symptoms* assessed by nurse or doctor	Patients n=5447		No parasites detected n=3998		Parasites detected n=1449		Odds Ratio	95% - CI[a]	p
	N	(%)	N	(%)	N	(%)			
Running Nose	3074	(56.4)	2466	(61.7)	608	(42.0)	0.44	0.39 - 0.50	<0.001
Elevated body temperature	2025	(37.2)	1143	(28.6)	882	(60.9)	1.97	1.86 - 2.09	<0.001
Blocked nose	762	(14.0)	631	(15.8)	131	(9.0)	0.53	0.43 - 0.64	<0.001
Skin rash	621	(11.4)	530	(13.3)	91	(6.3)	0.43	0.34 – 4.52	<0.001
Palmar pallor	477	(8.8)	218	(5.5)	259	(17.9)	2.80	2.38 - 3.30	<0.001
Skin abnormalities	344	(6.3)	268	(6.7)	76	(5.2)	0.77	0.59 - 1.00	0.051
Other skin problem	179	(3.3)	122	(3.1)	57	(3.9)	1.30	0.94 – 1.79	0.107
Malnourished condition	136	(2.5)	110	(2.8)	26	(1.8)	0.64	0.41 - 0.99	0.047
Prostration	115	(2.1)	61	(1.5)	54	(3.7)	2.49	1,72 - 3,62	<0.001
Fast breathing	88	(1.6)	72	(1.8)	16	(1.1)	0.60	0.35 - 1.05	0.074
Jaundice	82	(1.5)	35	(0.9)	47	(3.2)	3.79	2.44 - 5.90	<0.001
Breathing difficulties	60	(1.1)	50	(1.3)	10	(0.7)	0.54	0.27 - 1.08	0.084
Chest indrawing	59	(1.1)	46	(1.2)	13	(0.9)	0.77	0.41 - 1.44	0.426
Respiratory distress	58	(1.1)	40	(1.0)	18	(1.2)	1.24	0.71 - 2.17	0.443
Skin depigmentation	51	(0.9)	45	(1.1)	6	(0.4)	0.36	0.15 – 0.85	0.021
Deep Breathing	33	(0.6)	24	(0.6)	9	(0.6)	1.03	0.48 - 2.23	0.930
Pinch abdomen present	21	(0.4)	20	(0.5)	1	(0.1)	0.13	0.01 - 1.02	0.053
Cyanosis	1	(0.01)	0	(0)	1	(0.1)	0.91	0.09 - 8.79	0.938

* Patients could show multiple signs or symptoms
[a] CI: 95% Confidence interval

Table 6.2 Signs and symptoms and their association with P. falciparum parasitaemia in children

Signs and Symptoms* taken from history by child or legal guardian	Patients n=5447		No parasites detected n=3998		Parasites detected n=1449		Odds Ratio	95% - CI[a]	P
	N	(%)	N	(%)	N	(%)			
Report of fever	3859	(70.8)	2647	(66.2)	1212	(83.6)	2.61	2.23 – 3.04	<0.001
Nutrition changed to poor feeding	3659	(67.2)	2617	(65.5)	1042	(71.9)	1.32	1.16 – 1.50	<0.001
Cough	3081	(56.6)	2500	(62.5)	581	(40.1)	0.40	0.35 – 0.45	<0.001
Vomiting	1870	(34.3)	1275	(31.9)	595	(41.1)	1.48	1.31– 1.68	<0.001
Diarrhoea	1636	(30)	1264	(31.6)	372	(25.7)	0.74	0.65 – 0.85	<0.001
Drinking thirsty	425	(7.8)	284	(7.1)	141	(9.7)	1.40	1.14 – 1.74	0.001
Earpain	172	(3.2)	135	(3.4)	37	(2.6)	0.74	0.51 – 1.08	0.126
Convulsions	20	(0.4)	12	(0.3)	8	(0.6)	1.84	0.75 – 4.52	0.180
Generated variables [b]									
No malnourishment [c]	5311	(97.5)	3888	(97.2)	1423	(98.2)	1.55	1.01 – 2.38	0.047
No skin symptoms [d]	4687	(86.1)	3374	(84.4)	1313	(90.6)	1.79	1.47 - 2.17	<0.001
No gastrointestinalsymptoms [e]	2682	(49.2)	2019	(50.5)	663	(45.8)	0.82	0.73 – 0.93	0.002
No respiratory symptoms [f]	1639	(30.1)	1002	(25.1)	637	(44.0)	2.34	2.06 – 2.66	<0.001

* Patients could show multiple signs or symptoms; [a] CI: 95% Confidence interval

[b] For variables with an Odds Ratio of ≤0.83 bunched inverse variables were generated (e.g. no respiratory symptoms). These bunched variables exclude all variables of a group of symptoms (e.g. all respiratory symptoms) at the same time.

[c] To be positive for this (inverse) variable patients must not present *malnourished condition*

[d] To be positive for this (inverse) variable patients must not present *skin abnormalities*, *skin rash*, *skin depigmentation* and *other skin problem*

[e] To be positive for this (inverse) variable patients must not present *vomiting* and *diarrhoea*

[f] To be positive for this (inverse) variable patients must not present, *chestindrawing, breathing difficulties, fast breathing, running nose, blocked nose, cough*

3.4 Stepwise logistic regression

To estimate which of the variables were useful for prediction of *P.falciparum* parasitaemia, backward and forward stepwise logistic regression were performed. Only those variables were entered in the regression, that were found to be 'potentially predictive' according to the criteria mentioned above. Symptoms that met these criteria were *prostration, palmar pallor, jaundice, respiratory distress, other skin problem, report of fever, elevated body temperature, nutrition change to poor feeding, drinking thirsty, vomiting, no respiratory symptoms, no gastrointestinal symptoms* and *no malnourishment.*

Table 7. Result of forward and backward stepwise logistic regression with variables[a] from bivariate logistic regression potentially predictive for *P. falciparum* parasitaemia

Variable [a]	Odds Ratio	95% - CI[b]	p
Palmar pallor	3.06	2.49 – 3.78	<0.001
Elevated body temperature	2.82	2.47 – 3.23	<0.001
Report of fever	4.62	3.39 – 6.30	<0.001
Other skin problem	2.25	1.47 – 3.47	<0.001
Vomiting	1.33	1.16 – 1.52	<0.001
No respiratory symptoms	2.45	2.14 – 2.81	<0.001
No skin symptoms	1.97	1.51 – 2.56	<0.001
No malnourishment	1.71	1.08 – 2.76	0.025

Number of observations: 5447; Likelihood ration chi^2 : 843.64; p= <0.0001

[a]'Potentially predictive variables' were defined as variables providing an Odds Ratio of ≥1.20 and an occurrence in at least 1% of cases with present parasitaemia in the bivariate logistic regression. These variables were entered in the stepwise regression.
[b]CI, 95% confidence interval

Both parameter reduction methods revealed the same result, which is displayed in Table 7.Using a statistical cutoff of p ≥ 0.05 the following variables were found to be predictive for parasitaemia in the context of the logistic regression: P*almar pallor* with an OR of 3.06 (95%-CI: 2.49 – 3.78; p<0.001)*, elevated body temperature* with an OR of 2.82 (95%-CI: 2.47 – 3.23; p<0.001), *report of fever* with an OR of 4.62 (95%-CI: 3.39 – 6.30; p<0.001), *other skin problem* with an OR of 2.25 (95%-CI: 1.47 – 3.47; p<0.001), *vomiting* with an OR of 1.33 (95%-CI: 1.16 – 1.52; p<0.001), *no*

respiratory symptoms with an OR of 2.45 (95%-CI: 2.14 – 2.81; p<0.001), *no skin symptoms* with an OR of 1.97 (95%-CI: 1.51 – 2.56; p<0.001) and *no malnourishment* with an OR of 1.71 (95%-CI: 1.08 – 2.76; p<0.025).

These symptoms were then entered in the Classification and regression tree (CART).

3.5 Classification and Regression Tree (CART)

The CART was performed separately for the two age groups. In children between 2-12 months of age, the first decision was made by whether the patient was diagnosed with *palmar pallor* or not(Figure 6).

	n[a]	failure[b]	OR[c]
no → report of fever → no	255	5	0.14
no → report of fever → yes	553	56	0.73
elevated body temperature → yes → no respiratory symptoms → no	299	49	1.19
yes → no respiratory symptoms → yes	107	31	2.10
yes → no respiratory symptoms → no	61	20	2.38
yes → no respiratory symptoms → yes	29	19	4.75
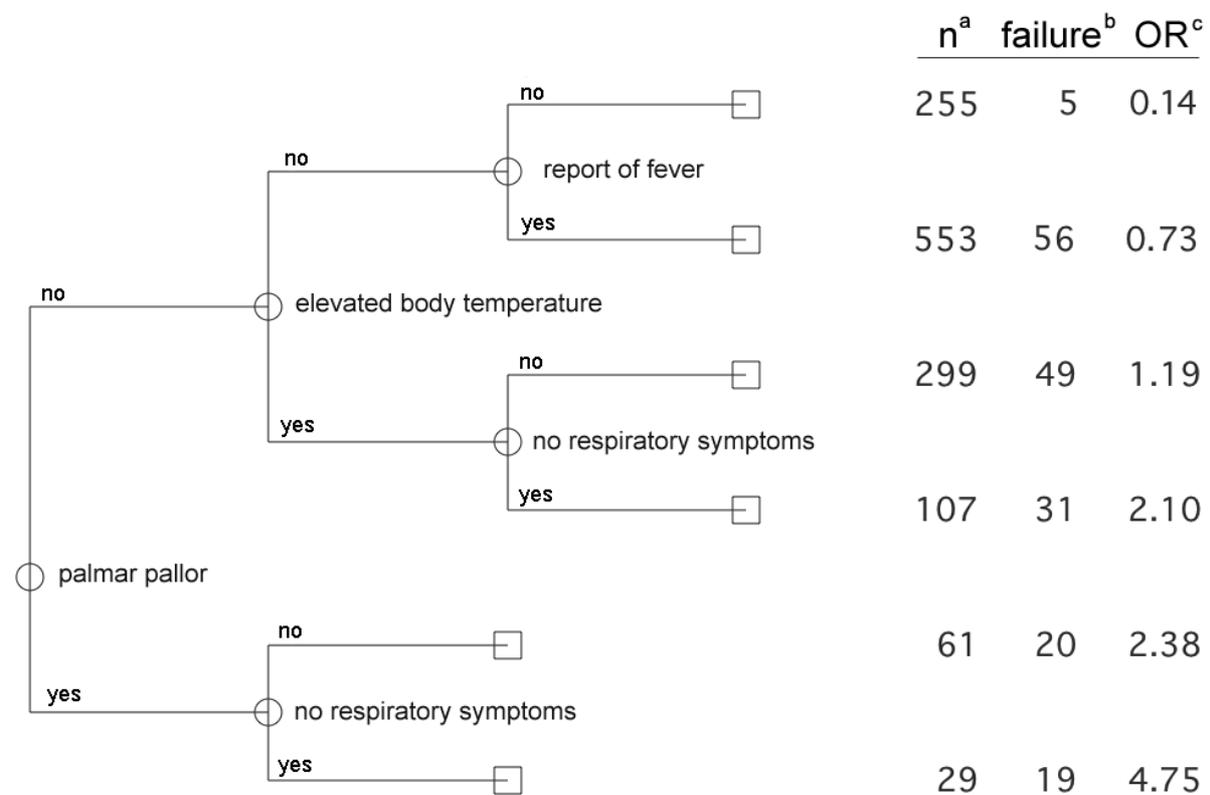
**Figure 6. CART for the prediction of P. falciparum positivity in children between 2 and 12 months of age (n=1304)**

Legend:
[a] Number of patients with therespective combination of variables given by the branches of the decision tree
[b] Number of patients positive for *P. falciparum*parasitaemia
[c] Odds Ratio for *P. falciparum*parasitaemiawith the combination of variables in comparison to all other combinations

In 19 children out of 29 who presented with *palmar pallor* but without respiratory symptoms (*no respiratory symptoms*=yes) a *P. falciparum* parasitaemia was detected (OR=4.75). To the contrary, patients without *palmar pallor*, without an *elevated body temperature* (≥ 37.5°C) on admission and without any *report of fever* in the last five days had an OR of 0.14 for parasitaemia.

Only 5 (2.0%) out of these 255 patients were tested positive for parasitaemia.

For those children, who had a positive *report of fever*, the likelihood of having parasitaemia was slightly higher with an OR of 0.73.

As also visible in Figure 6, not all of the variables that resulted from the stepwise logistic regression were used to create the decision tree of the CART-analysis. The variables *other skin problem*, *no skin symptoms* and *no malnourishment* were sorted out.


	n[a]	failure[b]	OR[c]
	591	39	0.22
	936	138	0.48
	403	88	0.71
	80	28	1.14
	353	93	0.86
	36	21	1.90
	175	82	1.53
	595	192	1.05
	299	142	1.55
	140	89	2.08
	535	357	2.18
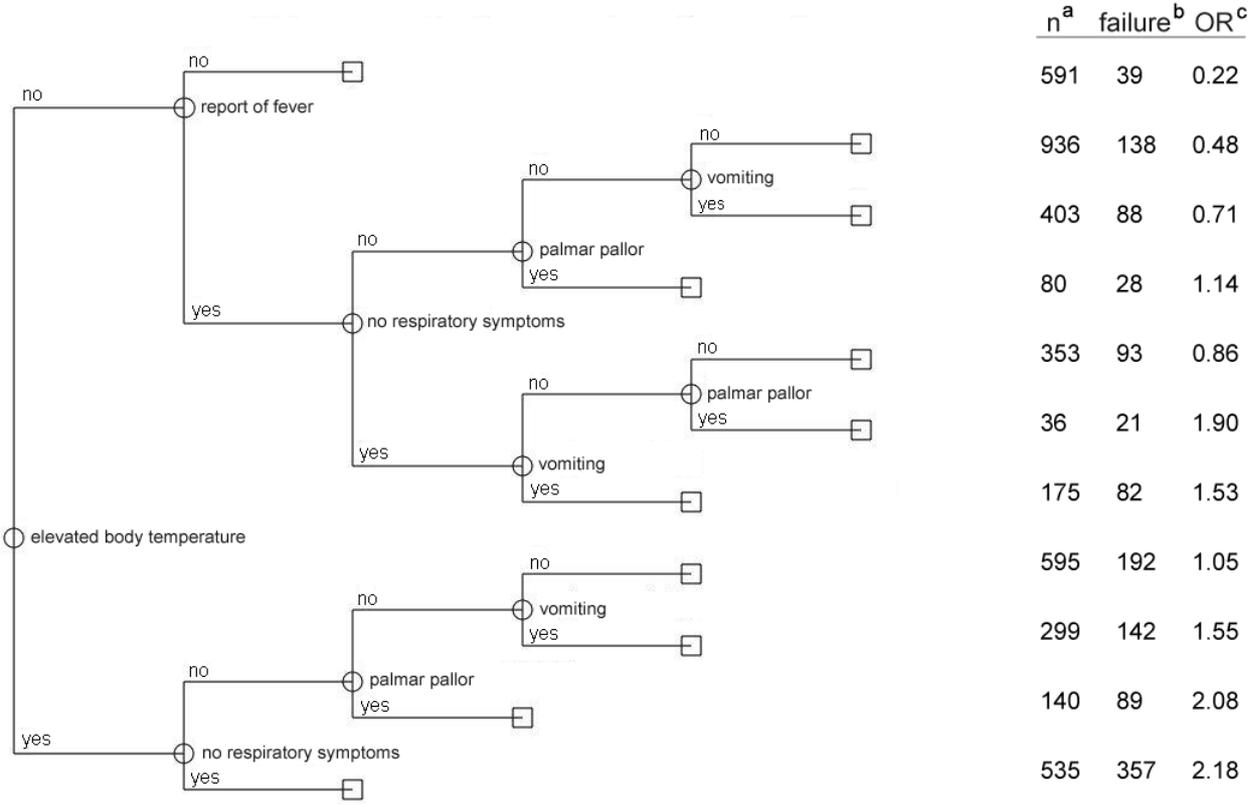
Figure 7. CART for the prediction of *P. falciparum* positivity in children between 12 and 60 months of age (n=4143)

Legend:
[a] Number of patients with therespective combination of variables given by the branches of the decision tree
[b] Number of patients positive for *P. falciparum*parasitaemia
[c] Odds Ratio for *P. falciparum*parasitaemiawith the combination of variables in comparison to all other combinations

The model used both variables indicating a fever, *elevated body temperature* and *report of fever*, respectively. Following the first branch of the CART and translated into clinical practice suggested to measure the body temperature of the patient first. If the body temperature was not elevated, the next question would have been for any history of fever, which adheres to common clinical proceeding.

Compared to the CART for children between 12-60 months several differences were obvious (Figure 7). While *palmar pallor* was strongly predictive for parasitaemia in children between 2-12 months of age, it caused only small differences of the OR in older children where *elevated body temperature* was the first decisive variable. The lowest likelihood for a parasitaemia was found in children without *elevated body temperature* and without *report of fever* (OR of 0.22 for malarial parasitaemia). On the other hand an *elevated body temperature* and absence of respiratory symptoms resulted in the highest risk for parasitaemia (OR 2.18).

In Table 8 data on the sensitivity and the specificity for the prediction of *P. falciparum* parasitaemia are shown for the five variables that remained in the CART analyses. In both age groups the proportion of children with *a report of fever* was larger than the proportion of children who were actually presenting with an *elevated body temperature* on admission.

Table 8. Sensitivity and specificity of symptoms for prediction of *P. falciparum* parasitaemia in different age groups

	Patients	2-12 months	12-60 months
ROF[a]	n (%)	1029 (78.9)	3539 (85.4)
	Sens. / Spec.	96.0/23.8	96.8/19.6
EBT[b]	n (%)	465 (35)	1569 (37.9)
	Sens. / Spec.	56.7/68.5	61.5/72.5
Palmar Pallor	n (%)	90 (6.9)	387 (9.3)
	Sens. / Spec.	21.7/95.5	17.3/94.2
Vomiting	n (%)	549 (42.1)	1321 (31.9)
	Sens. / Spec.	40/57.6	41.2/72.2
NRS[c]	n (%)	340 (26.1)	1299 (31.6)
	Sens. / Spec.	38.9/76	44.7/74.5

Note: Percentage refers to the total number of patients within each age group
[a] ROF=*report of fever;* [b] EBT=*elevated body temperature;* [c] NRS=*no respiratory symptoms*

In both age-derived models, *palmar pallor* had the highest specificity of all variables for predicting a malarial parasitaemia.

3.6 Palmar pallor and anaemia

Each participant of the study had to provide an EDTA-blood sample for a full blood count and thin or thick blood film. The distribution of the clinical diagnoses revealed anaemia as a common diagnosis and CART highlighted the predictive value of *palmar pallor* for *P. falciparum* parasitaemia. Thus haemoglobin (Hb) concentrations of the study participants were examined more closely. In Figure 8 the distribution of Hb-levels of patients of all ages irrespective any possible parasitaemia is shown.
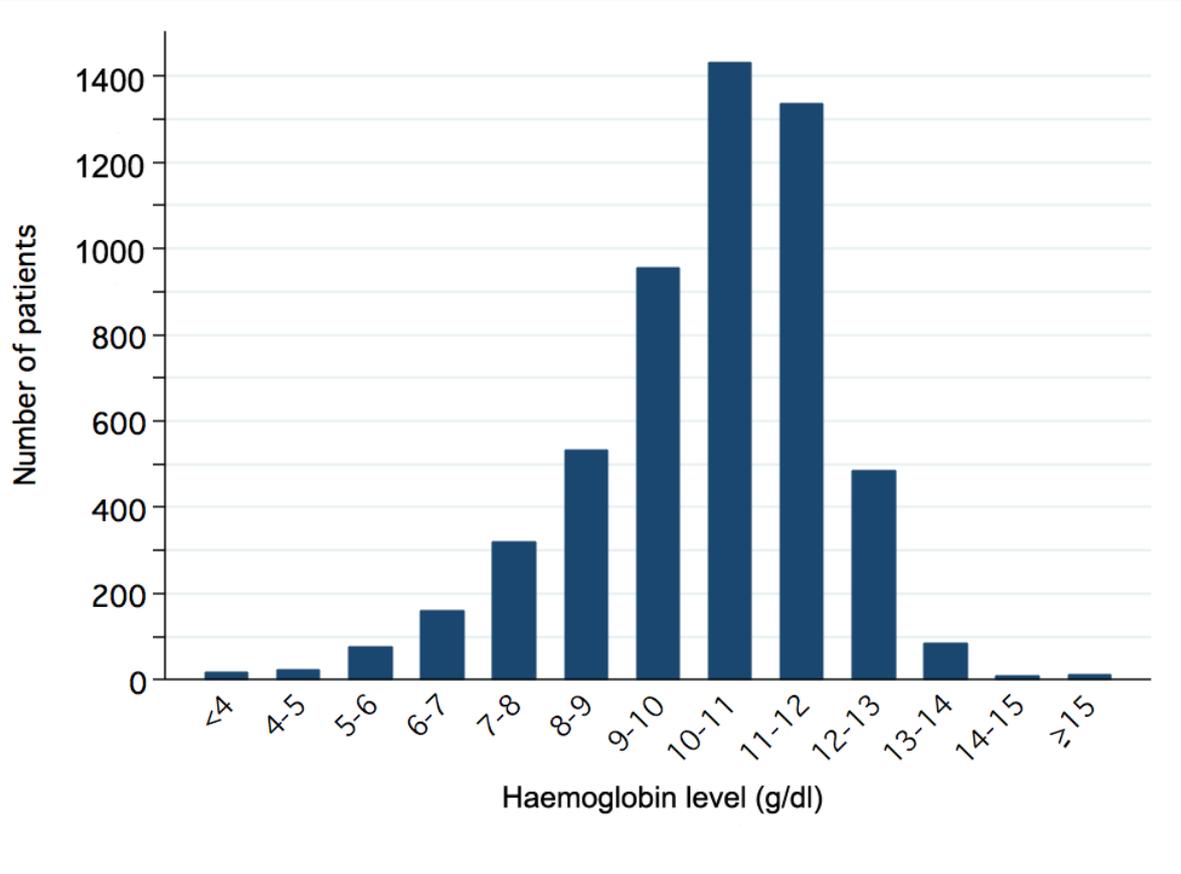

Figure 8: Distribution of Hb-levels in 5447 children of all ages (2-60 months)

Figure 9 displays the proportion of children infected with *Plasmodium falciparum* within each range of haemoglobin and revealed a clear dependency between the Hb-level and the infection status as children with parasitaemia had overall lower Hb-values compared to non-infected children.
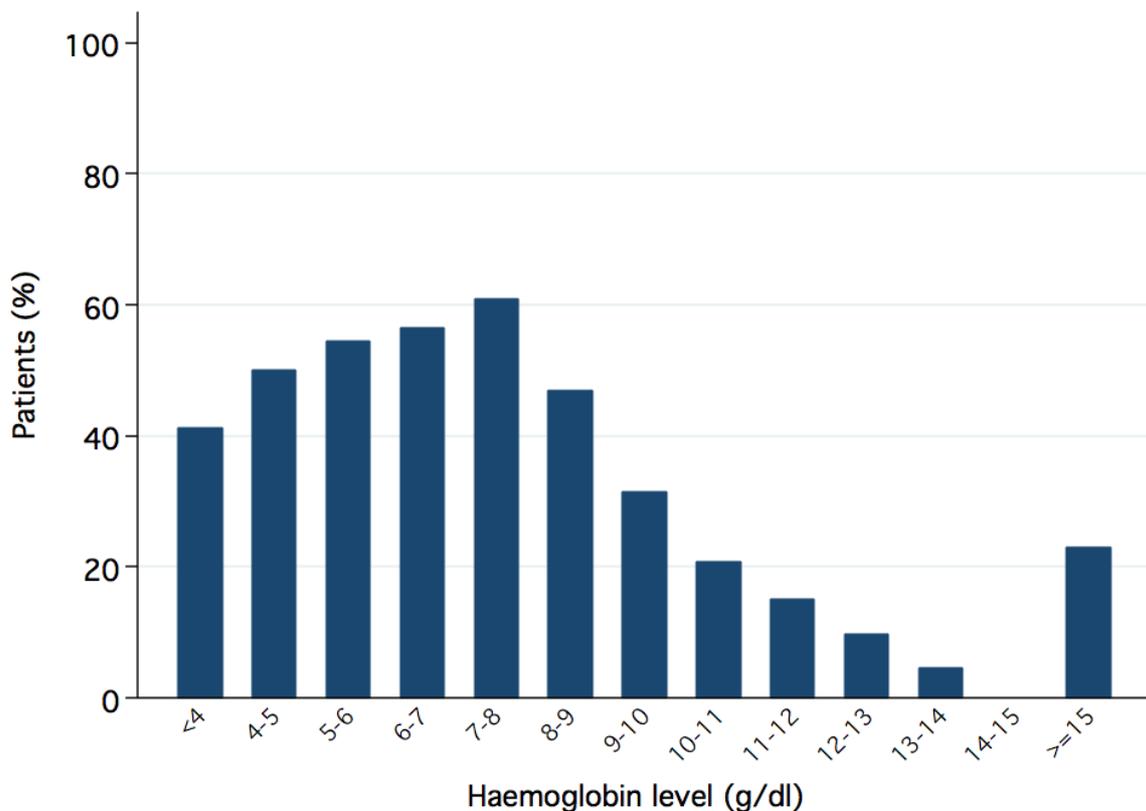
Figure 9. Proportion of patients with *P. falciparum* parasitaemia in relation to the distribution of haemoglobin levels

Solely with these figures it was possible to estimate that the majority of patients suffered from at least moderate anaemia. Setting a threshold of Hb ≤11 g/dl, Figure 10 shows the proportion of anaemic children in different ages and it is clearly visible that prevalence of anaemia was inversely correlated with the age of the patients. For this description children were stratified by age using strata of 10 to 12 months. While the proportion of children suffering from anaemia was 76.8% in children aged 2-12 months, it was 64.7% in children in the age between 24 and 36 months and 57.9% in children aged 48-60 months (Figure 10).

The symptom *palmar pallo*r was noticed in 477 (8.8%) of all patients. In the CART it was used as one of the major predictors for present parasitaemia.

To confirm that the predictive value of *palmar pallor* is applicable to clinical routine it was necessary to investigate whether the symptom *palmar pallor* was able to indicate decreased haemoglobin-levels. As expected, Hb-levels were indeed significantly lower for those children who were diagnosed with *palmar pallor* (Figure 11).
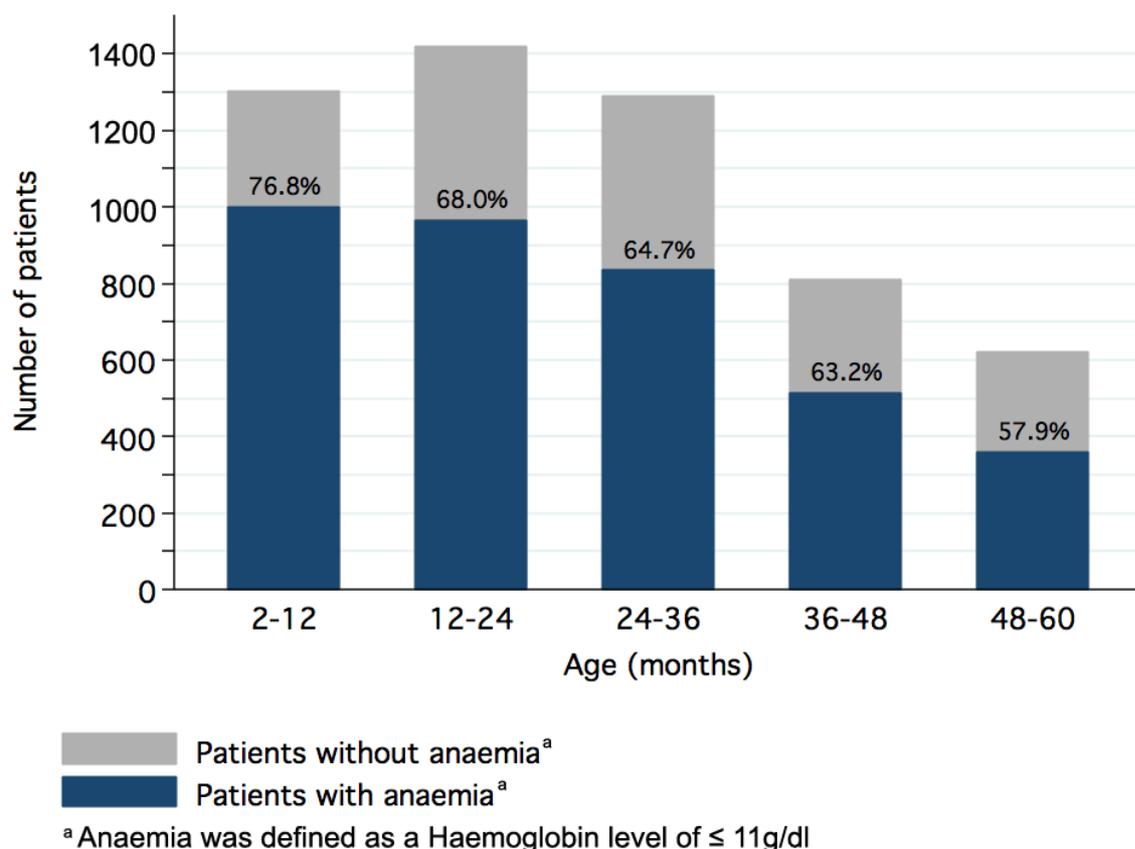
Figure 10. Proportion of mild anaemia in children of different ages

Children aged between 2 and 12 months had a mean Hb level of 7.7 g/dl if *palmar pallor* was present and 10.2 g/dl if not (p<0.001). Children aged between 12 and 60 months who were presenting with *palmar pallor* and those without had a mean Hb of 8.1 g/dl and 10.4 g/dl, respectively (p<0.001).

To discover potiential over-representation of *palmar pallor* due to the effect of multipe visits of single individuals a McNemar-test was used (Appendix Table A1). The McNemar-test showed no heterogeneity (p=0.342) for the symptom *palmar pallor* between patients who had only one visit to the OPD and those patients who came for more than one time.

Additionally, an alternative CART was created for both age groups containing only the first visit of every individual (Appendix Figure A1 & A2).
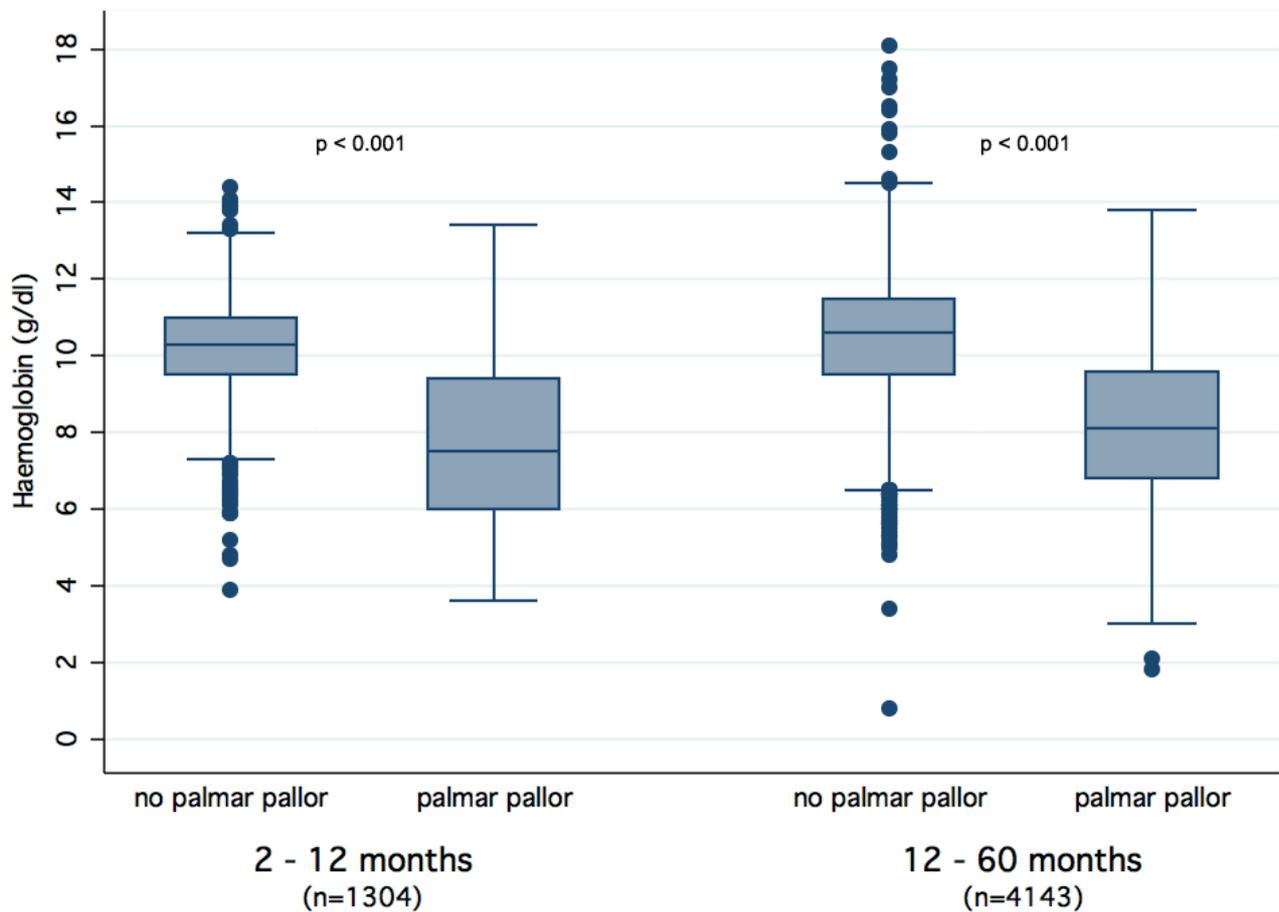
Figure 11. Distribution of haemoglobin levels in patients of different ages with and without palmar pallor

Legend:
p-value was calculated assuming a Student's t distribution

3.7 Classification and comparison of CART and IMCI

To gain more knowledge of the quality of the CART-model as a clinical algorithm it was compared to the Integrated Management of Childhood Illness – algorithm which is the main guideline in most African countries today.

For calculation of sensitivity. specificity, positive- and negative predictive value, a logistic regression model was created containing all variables used in the IMCI–algorithm and the CART, respectively. Both age groups were regarded separately. If applied to the population of children between 2-12 months of age in the present study, the IMCI algorithm would have resulted in a much higher sensitivity of 97.2% than the CART (6.7%), but would have remained unspecific in the prediction of parasitaemia with a specificity of 22.2% versus 99.6% in the CART (Table 9). The

positive predictive value was higher for the CART with 75.0% compared to 16.7% for the IMCI-algorithm.

For the older children between 12-60 months of age these relations were decreasing, as the IMCI-algorithm provided a sensitivity of 55.6% and a specificity of 73.4% vs. 37.7% and 91.4% for the CART, respectively.

Table 9: Classification and comparison of CART and IMCI algorithm for the sensitivity and specificity of the prediction of *P. falciparum* positivity

Age (months)	Model	Sensitivity (%)	Specificity (%)	PPV[c] (%)	NPV[d] (%)
2-12	IMCI-algorithm[a]	97.2	22.2	16.7	98.0
(n=1304)	CART[b]	6.7	99.6	75.0	87.0
12-60	IMCI-algorithm[a]	55.6	73.4	48.0	78.9
(n=4143)	CART[b]	37.7	91.4	65.8	76.9

[a] IMCI-algorithm for identification of children with malaria in high-risk areas: Fever by history or feeling hot/elevated body temperature of ≥ 37.5°C on admission and/or some or severe palmar pallor.
[b] CART: For calculation only those variables were used, which were included in the CART for the certain age group
[c] PPV = Positive predictive value
[d] NPV = Negative predictive value

Still, the CART provided higher positive predictive values (65.8% vs. 48.0%). Overall, the CART resulted in much higher specificities and higher positive predictive values for prediction of malarial parasitaemia in comparison to the IMCI-model, which provided higher sensitivities and negative predictive values.

3.8 Malnourishment

As interactions between a malnourished condition of children and malarial infections as well as anaemia in particular is well described in literature, nutritional deficiencies were recorded by weighting and measuring the height of every patient who attended the OPD. The results are provided as additional information in the appendix (Appendix Table A2 & A3). Z-scores for undernutrition (weight-for-age z-score) and wasting (weight-for-height z-score) were calculated. Underweight and wasting were defined as a z-score of -2 standard deviations (SDs) below the corresponding median. As visible in Table A2, undernutrition was overall equally distributed among

children between 2 months and 48 months of age. Between 10.2% and 12.0% of these patients fulfilled the definition of 'underweight for age'. Older children (48-60 months) were more affected by underweight, with a fraction of 16.6% with -2 SDs below the median.

Wasting, measured as a weight-for-age z-score of -2 SDs or below, rather affected the youngest children up to 2 years of age.

4. Discussion

The aim of the present study was to generate an age-derived clinical algorithm to predict *P. falciparum* parasitaemia in children between 2 and 60 months of age. By using a Classification and regression tree (CART) analysis simple clinical signs and symptoms should be discovered which may enhance the clinical decision making whether to treat patients suspected of having malaria or not.

As our results showed, the CART algorithm increased prediction specificity for malarial parasitaemia in comparison to a simple combination of single parameters. Specificity and positive predictive value for *P. falciparum* parasitaemia of the CART outperformed the IMCI-algorithm. The predictive value of the symptoms fever and *palmar pallor* was age-dependent. It was possible to show that the symptom *palmar pallor* is able to indicate significant lower haemoglobin - concentrations even in a population with a high prevalence of anaemia. Thus, use of pallor as a possible sign of anaemia due to malaria should be highlighted in health worker training.

However, despite the good specificity of the CART it is not possible to implement it in clinical routine due to a lack of sensitivity. By making treatment decisions only based on the application of the CART too many sick patients would have been missed. Therefore the use of clinical algorithms cannot replace laboratory diagnostics or rapid diagnostic tests (RDT). If these diagnostics are not available children suspected of malaria have to be treated presumptively.

To be able to provide reliable results the study was carried out in an area that is exemplary for a setting with high malaria transmission during the whole year. Gathered data from initially over 12.000 enrolled patients was condensed to a dataset, which is highly consistent regarding all analyzed variables. Apart from earlier studies, we only assessed children between 2-60 months of age attending an OPD for any episode of illness within the study period. This age group is the one at highest risk of dying due to malaria (Eliades et al. 2006, World Health Organization 2008a).

4.1 Parasitaemia and Malaria

To gain more knowledge about the role of the symptom fever for the prediction of malarial parasitaemia it was decided not to use 'fever' or a 'history of fever' as an inclusion criterion. Accordingly, the primary outcome was defined as *Plasmodium*

falciparum parasitaemia of any density instead of 'clinical malaria', which is commonly defined as presence of parasitaemia and 'fever' or a 'history of fever' (Bojang et al. 2000, Muhe et al. 1999, Redd et al. 1996).

With an overall prevalence of 26.6%, we measured a comparatively low level of *Plasmodium* parasitaemia (Bouyou-Akotet et al. 2009, Gay-Andrieu et al. 2005, Guinovart et al. 2008, Ronald et al. 2006). A study conducted in northern Ghana in the year 2006, showed a prevalence of 82% in the rainy season and 75% in the dry season (Ehrhardt et al. 2006). Researchers in Tanzania even found over 90% of children aging one to four years infected with *P. falciparum* parasites and a study from Togo revealed a prevalence of 62.2% (Eliades et al. 2006, Smith et al. 1993). Although a cross-sectional survey conducted in Kumasi, Ghana, underlined that malaria transmission can vary widely across certain areas (Ronald et al. 2006), two other reasons may explain the relatively low prevalence in the present study: First, we enrolled all children attending the OPD, disregarding any prerequisites. If we had used any form of fever definition as an inclusion criterion the prevalence would have been much higher. Secondly, there is increasing evidence of declining malaria transmission in Africa in the last years (World Health Organization 2010b, Bouyou-Akotet et al. 2009b, Ceesay et al. 2010). These reports may not at least reflect the efforts of the WHO and the affected countries themselves to reduce the impact of malaria by improving overall health capacities (e.g. distribution of long-lasting insecticidal nets (LLINs)) over the last decade (World Health Organization 2008d).

Regarding the age-dependent prevalence of *P.falciparum* parasitaemia our results were in line with previous findings. Due to the increasing susceptibility after the first four to six months of life, prevalence of malarial infections is also rising in older children (Eliades et al. 2006).

As visible in Figure 4 and 5 the proportion of patients with parasitaemia was higher than the proportion of patients with defined 'clinical malaria', except in children between 2-12 months of age. This gap was owed to the significant number of patients that presented to the OPD without an *elevated body temperature* and without a *report of fever.*

4.2 Methodology

For the statistical analysis a patient was defined as an individual visiting the OPD. To avoid inclusion of an individual with the same episode of illness for a second time a

minimum latency of 14 days since the last visit was set. Follow-up visits within these 14 days were treated as reviews and were not included in the analysis.

Assessment of the children was performed by three pediatricians and several study nurses. As the entire study was conducted over a period of almost three years it was implemented in daily clinical routine. Thus, it was not possible to assess every patient twice to get information about possibly existing inter-observer bias. To minimize confounding effects during the assessment we used a case report form with pre-defined criteria. However, it was possible for the doctor to state additional information, which was later transformed into binary variables.

The distribution of diagnoses made by the assessing doctors (Table 5) reflected current statistics of UNICEF (Unicef 2012). Usually, laboratory results should have been available for the doctor before he made the diagnosis. But as the big difference between the number of patients with the clinical diagnosis 'malaria' (50.6% of all children) and the number of patients with detected *P. falciparum* parasitaemia (26.6%) demonstrated a high proportion of patients was diagnosed false positive.

The most likely explanation for this discrepancy may be the non-availability of the test results at the time of making the diagnosis. But, however, non-adherence to negative test results also has to be taken into account. Earlier investigations of the adherence to negative test results of RDT's in this context have shown that physicians tended to ignore negative test results and treated patients with anti-malarials assuming the test result to be wrong (Ansah et al. 2010, Bisoffi et al. 2009, Kyabayinze et al. 2010). However, we used parasite microscopy and we assume that the physicians in Agogo generally trusted the work of their laboratory, which worked on a high standard level.

4.3 Predictive signs and symptoms for *P. falciparum* parasitaemia

The first step of the analysis was to verify, whether single independent variables could be helpful to predict a *P.falciparum* parasitaemia in children who were presenting to the OPD. In the present study these independent variables were mainly simple signs and symptoms, which can be assessed without any special medical equipment. Putting these signs in bivariate logistic regression, Odds Ratios for the outcome *P. falciparum* parasitaemia were calculated.

As already mentioned above, a *report of fever* and an *elevated body temperature* were also treated as independent variables. Regarding Table 5.1 and 5.2 it is obvious that we particularly assessed symptoms, which reflect less severe

conditions. Symptoms like *unconsciousness*, *convulsions* or *cyanosis* were overall less frequently observed, which may be explained by the study design and setting: All children attending the OPD, for instance also children who suffered from minor illness (e.g. a common cold), were included in the calculation. Further the population of Agogo is used to medical research in the Presbyterian Hospital, which could have resulted in enhanced health care seeking behavior. From this point of view it is likely, that most of the enrolled patients with parasitaemia were assessed before their condition became worse.

While some prior studies dealt with symptoms in critically ill children in order to predict severe illness or fatal outcome (Dzeing-Ella et al. 2005, Marsh et al. 1995, Mockenhaupt et al. 2004), others were solely focused on clinical features predicting malaria (Bojang et al. 2000, Muhe et al. 1999, Mwangi et al. 2005). Results of the latter were not entirely consistent as their predictive value depended on certain settings:

In those studies where it was not part of the definition of the outcome ('clinical malaria') or an inclusion criterion, fever or a history of fever was strongly associated with malarial parasitaemia (Genton et al. 1994, Redd et al. 1996). In the present model fever was still used as a substantial predictor but showed i) different predictive value across the age groups (lower in young children, higher in older children) and was ii) outperformed by the symptom *palmar pallor* especially in young children. The presence of respiratory symptoms like *cough* or *running nose* commonly expresses upper respiratory tract infections, which can be easily accompanied by fever and was consequently not found to be associated with malarial parasitaemia (Muhe et al. 1999, Mwangi et al. 2005). To the contrast signs like *respiratory distress* or *fast breathing* were recognized as signs of 'severity of illness' and were more often associated with a positive malaria parasite status (Muhe et al. 1999, Schellenberg et al. 1999). Particularly *respiratory distress* is discussed to be an indicator for metabolic acidosis and/or developing heart failure due to severe malarial anaemia (Bojang et al. 2000, Marsh et al. 1995).

In the present study mild respiratory symptoms were indeed some of the most frequent since 56.6 % of patients were presenting with *cough* and 56.4% with a *running nose*. Bunched together in the variable no respiratory symptoms it was possible to use the absence of respiratory symptoms as a predictor for malarial parasitaemia or, in other words: Presence of respiratory symptomns reduced the

likelihood of the presence of parasitaemia and increased the likelihood of presence of an respiratory disease, which led to the OPD visit.

For other symptoms findings varied between different studies: *Dehydration* and *poor feeding* were found to be negatively associated with malaria in a study of Muhe et al. in 1999, while other researchers even highlighted the role of dehydration (Schellenberg et al. 1999). A poor nutritional status was described to be positively correlated with malaria (Muhe et al. 1999, Schellenberg et al. 1999).

Enlargement of spleen and/or liver was found to be predictive in most of the studies (Bojang et al. 2000, Muhe et al. 1999, Olaleye et al. 1998) but research also showed that correct assessment of spleno- and hepatomegaly could be difficult for health workers (Grover et al. 1993). For this reason we waived to use these signs.

At the present study site we assessed several symptoms that were not covered by prior studies mentioned above (e.g. *earpain, skin symptoms*). Furthermore some of the signs and symptoms in our analysis, like respiratory symptoms and skin symptoms initially provided ORs that seemed to have a protective value for a diagnosis of malarial parasitaemia. Given this, presence of skin symptoms (e.g. skin rash) was an expression of another conditions (e.g. measles) and, in turn, absence of this symptom in a febrile child could guide the way to a parasitaemia. Thus, for these variables inverse variables were created to keep them in the analysis as a potential predictor.

From the initial 30 covariates in Table 6.1 and 6.2, which were put in bivariate logistic regression as a sort of prescreening to generate a set of variables that provide a certain 'a priori likelihood ' to predict *P. falciparum* parasitaemia, 13 satisfied the criterion of an Odds Ratio of ≥1.2 and an occurrence of ≥1% in parasite positive patients. These were handled as potentially predictive. As 13 variables were still too many to provide a useful model that is easy to interpret we used the stepwise logistic regression as a method of 'assessment of fit' (Lemeshow and Hosmer 1982). This parameter reduction technique enabled us to develop a set of variables where only those variables were included that have the best fit to each other (Table 6). To ensure consistency stepwise logistic regression was calculated in both directions – as forward and backward logistic regression. Both calculations revealed the same set of variables (Table 7), which were then entered in the Classification and Regression Tree.

4.4 Classification and Regression Tree (CART)

In contrast to earlier studies, where multivariate logistic regression was used to trace for predictive variables for malaria (Chandramohan et al. 2001, Muhe et al. 1999, Mwangi et al. 2005), we used a Classification and regression tree – analysis, which is a completely different approach. Using multivariate logistic regression alone it is not possible to explore the relationship between different independent variables and their relative importance (Thang et al. 2008). Logistic regression is also not able to rank risk factors according to their importance. Therefore, we decided to use the CART, which is a non-linear and non-parametric alternative for regression and classification problems (Thang et al. 2008). Gaudart et al. described the nature of CART as "fitted by binary recursive portioning of a multidimensional covariate space in which the dataset is successively split into increasingly homogeneous subsets until a specified criterion is stratified" (Gaudart et al. 2005, page 2).

Regression trees are a hierarchical attempt that provides a more flexible relationship between variables and is even able to deal with multi-collinearity and outliers. It is also possible to handle missing values as own subgroups and therewith separate them from further analysis (Saegerman et al. 2004). However, we did not use the latter, as all patients with missing values for a certain set of variables were excluded from our dataset. CART is easy to interprete even for non-statisticians and has successfully been proven applicable in different medical studies (Bachur and Harper 2001, Crichton et al. 1997, Zhang et al. 1996). Nevertheless some researchers also mentioned disadvantages of the method, mainly caused by the strong hierarchical orientation. Speybroeck doubted that it would be possible to discover simple decision rules by "tree-growing". Furthermore they raised the concern that subgroups created by the CART may be hard to interpret and net effects of independent variables are frequently not discovered (Speybroeck 2011).

For the calculation of the CART in the present study the variables *palmar pallor, elevated body temperature, report of fever, other skin problem, vomiting, no respiratory symptoms, no skin symptoms* and *no malnourishment* were entered. The contradictory selection of the conflictive variables *other skin problem* and *no skin symptoms* was abolished as the CART did not use one of these variables.

Instead, CART used the variables *palmar pallor, elevated body temperature, report of fever, no respiratory symptoms,* and *vomiting* for creating a decision tree for both age groups. The model for patients between 2-12 months of age is obviously less

complex than the model for patients between 12-60 months of age, which may partly be owed to the higher number of patients in the second group leading to a greater variety of clinical presentations. Regarding the structure of the models they are clearly orientated on clinical decision making as, for instance, in the model for the older children the elevated body temperature should be assessed first followed by the question for any report of fever. Although age dependent differences of certain variables are quite obvious, there is also evidence throughout both models that the variables alternate in their predictive value disregarding the age of patient they are used for. Whilst the presentation of palmar pallor was causing big differences in the risk of having malarial parasitaemia especially in younger patients, the variable vomiting was only responsible for little changes of the Odds ratio. Nevertheless, vomiting reveled a relatively high sensitivity and specificity for the prediction of parasitaemia (Table 8).

4.5 Age-dependent differences in the predictive value of fever and palmar pallor

The main difference between the two age-derived CART models was enunciated in the predictive value of the symptoms *report of fever,elevated body temperature* and *palmar pallor*. While the predictive value of fever was increasing with the age of the patients, the variable palmar pallor was the most decisive sign in the youngest children between 2-12 months of age. For those children positive assessment of *palmar pallor* and absence of any respiratory symptoms sufficed to result in an Odds ratio of 4.75 for having malarial parasitaemia (Figure 6). In the CART for the older children *palmar pallor* was responsible for only small differences of the Odds ratio and it was not involved in every single decision.

To gain more knowledge about the predictive role of the symptom fever in the diagnosis of *P. falciparum* malaria we used two definitions of fever. Using the history of fever given by the legal guardian of the child has been proven to lead to significant overdiagnosis if it equals the case definition of malaria in high transmission areas (Okiro and Snow 2010, Reyburn et al. 2004, Zurovac and Rowe 2006a). In this study legal guardians of 66.2% of children without malaria parasites in their blood reported any episode of fever or feeling hot. Exclusive application of fever as decision criterion for treatment would have consequently resulted in a false treatment of 2647 children with anti-malarials. Beside the economic impact of unnecessary medication and the

indisputable risk of overseeing other underlying causes of the fever, when focusing on the diagnosis malaria, this strategy has its justification only under certain circumstances. Thus, to date only health workers in settings where no laboratory or RDT–diagnosis is possible are asked to treat presumptively (World Health Organization 2010d).

Statements about the validity of the symptom *report of fever* are difficult. Studies have shown that mothers are indeed able to detect an elevated body temperature in their children with sensitivities up to 74%, which makes it reasonable to consider a *report of fever* as a reliable information (Wammanda and Onazi 2009, Ye et al. 2007). An onset of fever has also frequently shown to be the trigger for seeking help from health facilities (McCombie 1996, Molyneux et al. 1999). On the other hand clear definitions should ensure that the term fever is not ambiguously used as studies also discovered that in some societies the term fever is used as a synonym for "a general state of poor health" (Einterz and Bates 1997). To objectify the presence of fever every child's temperature was measured on admission and the variable *elevated body temperature* was created. As visible from Table 4 there is a noticeable gap between the numbers of patients with a *report of fever* and an *elevated body temperature* on admission. This may be particularly owed to the broad use of antipyretics, as 73.2% of all children enrolled in this study received Paracetamol prior to their visit.

This confounding effect of antipyretic use makes it difficult to rely only on an *elevated body temperature* on admission and in turn necessary to assess, whether a child had a prior history of fever. The CART for children between 12-60 months of age reflects this attempt in a solid way. In settings where high numbers of parasite positive patients are assumed it is necessary to use the most sensitive variable to catch as many sick patients as possible. Table 8 underlines that a *report of fever* satisfy this criterion, although comparison of the specificity between *elevated body temperature* and *report of fever* indicates that a significant number of patients with a positive *report of fever* had a fever of other origin or was false positive for this variable.

Apart from the validity of the variables indicating a fever themselves some raised the question, if fever can serve as an indicator for malaria any longer (D'Acremont et al. 2009) as prevalences of *Plasmodium falciparum* transmission intensity have been reported to decline in several parts of Africa (Hay et al. 2009, World Health Organization 2008d). This would consequently result in larger proportions of other

conditions possibly accompanied with similar symptoms like malaria (Reddy et al. 2010, Ukwaja et al. 2011), needing different diagnostic and treatment strategies (Brent et al. 2006) (see section 4.7).

The second symptom with a clear age-dependency in this study was *palmar pallor*. The CART for children aged between 12-60 months revealed this symptom as the most decisive variable in this age group. Pallor due to anaemia is a common clinical sign in SSA (Desai et al. 2005, Ronald et al. 2006, World Health Organization 2008b), in the present study it was noticed in 8.8% of all cases.

Defining anaemia by setting a haemoglobin (Hb) cut – off of ≤11.0 g/dl (Quinto et al. 2006), up to 76.8% of patients in the group of children aged between 2-12 months were found to be anaemic according to their full blood count (Figure 10). Compared to other studies this appears to be a very high prevalence (Akhwale et al. 2004, Magalhaes and Clements 2011). Various causes have been described to be involved in the development of anaemia. While iron deficiency was frequently described to be the main contributor to anaemia in west Africa (Kraemer and Zimmermann 2007, World Health Organization 2001), infectious diseases, malnutrition and haemoglobinpathies are other common causes (Bates et al. 2007, Means 2000, Morris et al. 2006). The mechanism of anaemia due to infectious diseases mainly includes three processes: Shortened red cell survival, impaired Erythropoetin (EPO) production and impaired mobilization of the reticuloendothelial system to iron stores (Cartwright 1966, Means 2000). In acute malaria anaemia is a hybrid between predominant haemolytic anaemia and anaemia of infectious diseases as increased cytokine production and impaired EPO response also seem to be involved (el Hassan et al. 1997).

Nutritional deficiencies are known to play an important role in childhood anaemia too: Verhoef and colleagues could illustrate with their findings that malnutrition is associated with more severe occurrence of malarial anaemia (Verhoef et al. 2002). Desai et al. also made stunting and concurrent fever responsible for significantly lower mean haemoglobin levels (Desai et al. 2005). Except in children aged 48-60 months, where the percentage was higher, the proportion of children suffering from undernutrition was around 10% in the present study, which is relatively low compared to figures from other areas (Beiersmann et al. 2012). De Onis et al. even forecasted an estimated average increase of underweight in sub-saharan Africa from 24.0% to

26.8% between the years of 1990 and 2015 (de Onis et al. 2004). The fact that children, especially in tropical areas, of West Africa are often less effected by severe undernutrition may be due to the relatively good access to high-carbohydrate nourishment (e.g. yams, cassava, rice, corn). However, there is still a lack in micronutrients like iron, folate, zinc, the B vitamins and calcium. This consequently results in specific malnourishment of these substrates and is probably an explanation for the large fraction of anaemia (e.g. through a lack of iron and B12 vitamin) in our population. In bivariate logistic regression only one variable reflecting the status of chronic undernutrition was related to the outcome. A malnourished condition was associated with *P. flaciparum* parasitaemia with an OR of 0.64, the inverse variable in turn resolved in an OR of 1.55. That said, the variable *nutrition change to poor feeding*, which was predictive for parasitaemia with an OR of 1.32, must be seen as an expression of short term change of behavior in a sick child in general.

Studies concerning a relationship between malnourishment and malaria susceptibility gave mixed results in the past. Deen et al. stated in his paper, that neither wasting nor undernutrition influenced susceptibility to malaria, but children, who were stunted experienced malaria attacks more frequently, than children, who were not stunted (Deen et al. 2002). In contrast to this, an earlier study, conducted by Genton et al. found, that stunting protects against falciparum malaria. The authors explained their findings with an improved ability of malnourished children to produce certain cytokines in response to malaria antigens (Genton et al. 1998). After all, it is commonly undisputed that children with severe protein malnutrition have an significantly increased risk to die from malaria, compared to those children, who are in good nutritional state (Caulfield et al. 2004, Mockenhaupt et al. 2004, Muller et al. 2003).

In the setting of this study, *palmar pallor* was assessed by experienced pediatricians. Investigation of full blood counts showed that the sign *palmar pallor* could predict significant lower levels of hemoglobin even in a population with a very high prevalence of anaemia. Indeed, children with *palmar pallor* had significantly lower Hb-levels than those who were not diagnosed as being pale on their palms (Figure 11). The quality of the palms as a site to reliably predict pallor was confirmed in earlier studies. Chalco et al. showed that the best predictor for a level of haemoglobine ≤11 g/dl was palmar pallor, conjunctival pallor and nailbed pallor (in

descending order). For an Hb ≤8 g/dl only plamar pallor gained sensitivities of >80% (Chalco et al. 2005).

Previous studies showed diverse results on the ability of health workers to recognize pallor (Chalco et al. 2005, Kalantri et al. 2010, Strobach et al. 1988). Health workers in high malaria transmission areas had less difficulties (Weber et al. 1997).

As we regarded patient visits instead of individuals in this study, it was possible for an individual to attend the OPD for several times within the study period. To exclude any confounding effect due to multiple visits of single individuals with a personal predisposition for anaemia leading to an overrepresentation of the symptom *palmar pallor*, multiple visits of individuals were also analyzed. The McNemar-test showed no heterogeneity (p=0.342) for the symptom *palmar pallor* between patients who had only one visit to the OPD and those patients who came for more than one time (Table A1). An alternative CART that included only the first visit of every individual also showed no deviance from the original models (Figure A1 and A2).

In conclusion, we could show that *palmar pallor* is a reliable sign to predict *P. falciparum* parasitaemia in the context of an algorithm, especially in children between 2-12 months of age. The sign is eligible to detect Hb levels of ≤11 g/dl even in a population with a high prevalence of anaemia. It is therefore reasonable to introduce *palmar pallor* to health workers as a sign not only for anaemia but also for malarial parasitaemia. Because of a lack in sensitivity it is however not possible to use the sign to rule out a parasitaemia. The high prevalence of anaemia in the study population may be due to a combination of malnourishment of micronutrients and malaria infections. Although it was not possible to measure the levels of iron, B12 vitamin etc., it is unlikely that *P. falciparum* parasitaemia (with a overall prevalence of 26.6%) is solely responsible for such a high prevalence of anaemia.

4.6 Comparison of CART to IMCI and other algorithms

The subjacent aim of the quest for the best clinical algorithm is the biggest challenge at a time. An optimal algorithm should reliably detect sick patients with high sensitivity. It should also enable the physician to distinguish between different illnesses, i.e. provide a high specificity. In addition it should be easy to use, consist of simple criteria and minimize the possibility of inter-observer bias.

Research in the context of malaria has shown that most attempts could not fulfill these high standard requirements. Either, the algorithms lacked in sensitivity or specificity or the signs applied were too difficult to assess, i.e. necessary technical equipment was not available. Chandramohan et al. gathered data from articles about ten algorithms for diagnosing clinical malaria (Chandramohan et al. 2002). With a closer look on these algorithms, it is noticeable, that the majority of researchers worked with a complex set of signs and symptoms. Most of them used logistic regression models to trace for predictive symptoms.

Latest published data about trials of establishing algorithms dates from 2002 and 2005 (Chandramohan et al. 2002, Mwangi et al. 2005). Both of these studies, which were conducted in areas of medium and seasonal *Plasmodium* transmission, contained of a set of 18 to 19 signs and symptoms that have to be assessed and calculated for application of the algorithm.

Mwangi and colleagues calculated a score for each individual using a simple count of symptoms that were found to be predictive. For children under five years of age, the score that gave the best values for sensitivity and specificity was found to be 10. Estimated sensitivity and specificity were 79.6% and 55.5% respectively. The PPV was calculated to be 63.6%, the NPV was 73.6% (Mwangi et al. 2005). Although these values partly outperformed the IMCI – algorithm, it is important to bear in mind that transmission of a complex algorithm such like this is likely to cause problems if used by health workers.

Bojang et al. (Bojang et al. 2000), who worked with an algorithm established by Olaleye et al. in 1998 (Olaleye et al. 1998), gained a sensitivity of 88% and a specificity of 62% using an algorithm, which requires presence of 7 predictive variables. The pattern of signs and symptoms was similar to the study of Mwangi.

The comparability of most of the studies is limited as research settings differ in methods, endemicity of the study area, age of the study population and outcome. Some studies were carried out with only few participants (Gay-Andrieu et al. 2005, Yacoub et al. 2005), amother study was a meta-analysis (Chandramohan et al. 2002). Although less affected by malaria due to matermnal protection, there were more studies conducted for infants < 2 months (Jeena et al. 2008, Yeboah-Antwi et al. 2008, Young Infants Clinical Signs Study Group 2008), than for children between 6 months and five years of age.

The Integrated Management of Childhood Illness Programme is the best known and most widespread approach for improving childhood health. In its Guidelines for the treatment of malaria the WHO until 2010 recommended immediate presumptive treatment of all patients suspected of malaria until 2010 (World Health Organization 2010c). Following this approach all children living in high transmission areas presenting to a health facility with an elevated body temperature or a history of fever were treated with antimalarials. In view of the high risk of rapid death due to malarial parasitaemia particularly in young children this approach was the most sensitive and safest way to deal with high prevalences of malarial infections in endemic areas. Mwangi et al. compared applied the IMCI - algorithm to their population and calculated a sensitivity and specificity for the diagnosis of malaria of 58.4% and 67.6%, respectively (Mwangi et al. 2005). Beyond the population of the present study IMCI would have resulted in a much higher sensitivitiy of 97.2 % for children aged 2-12 months and 55.6% for children aged 12-60 months, respectively. Especially in the group of the youngest children application of the IMCI-model would have resulted in only very few patients with present parasitaemia who were mistakenly not treated.

The vast discrepancy in sensitivity of 38.8% between the study of Mwangi et al. and the present study can be regarded as an indication for the aggravated comparability of certain studies due to substantially different settings. In high – transmission areas the IMCI would have resulted in much higher sensitivities as calculated by Mwang and collegues. Further, they conducted a household – survey where health workers catched symptomatic children at home, all participants lived in areas of low malaria transmission (10-53 infective bites per person per year) and fever was used as inclusion criteria.

In the CART five signs and symptoms were applied to both age groups. It was possible to confirm that higher developed algorithms are able to provide much higher specificities, when compared to the IMCI-algorithm but lack in sensitivity. For children between 2 and 12 months IMCI gave a sensitivity of 97.2% and a specificity of 22.2%, CART provided 6.7% and 99.6%, respectively.

The symptom *palmar pallor* revealed high age-dependent specificity but as well as for the CART-models the sensitivity was very low. Vital for the decision of the health workers and the health of the patient are the predictive values, which are dependent on the prevalence of the outcome. With decreasing prevalence of malaria the negative predictive value will increase and the positive predictive value will decrease

given the same sensitivity and specificity of the algorithm. Compared to the IMCI-algorithm the positive predictive value (PPV) was higher with 75.0% for children between 2-12 months and 65.8% for children between 12-60 months when applying the CART.

However, comparison of the CART to IMCI-model may be aggravated as the outcome in the present study was *P. falciparum* parasitaemia and IMCI mainly targets 'clinical malaria'.

Concluding the principal findings it is obvious that the hierarchical CART-based decision algorithm is able to increase the prediction specificity for malarial parasitaemia in comparison to a simple combination of single parameters. Despite of a good specificity of the best model, a significant proportion of children with malaria would have been missed with this clinical algorithm. Particularly beyond the youngest children who are at highest risk of dying rapidly, significant false negative rates are unacceptable. Wherever possible laboratory confirmed diagnosis or use of RDTs should be striven for. In case of unavailability of these diagnostics, presumptive treatment of all children suspected of having malaria is necessary.

4.7 Clinical relevance of algorithms and future challenges in malaria diagnostic

Clinical algorithms are only one potential part of the clinical process in the management of a certain disease or condition. Thus, bringing the evaluation of the practical use of clinical algorithms in context with the intended diagnosis and treatment strategies is necessary. For children with an episode of fever who live in high transmission areas presumptive treatment for malaria was promoted for a long time by the WHO. As already stated above, this strategy fits the comprehensible desire for optimal protection of those children who are at the highest risk of dying.

During the last years malaria transmission rates are reported to decline and reliable rapid diagnostic tests have been made widely available and can adequately replace parasite microscopy. Consequently, the WHO changed its recommendation for malaria treatment to a general "test-and-treat" strategy in 2010 (Graz et al. 2011): Only in settings where parasitological confirmation is not possible, presumptive treatment should be still performed.

At which point are clinical algorithms now interfering with this policy change?

Responding to this question requires a closer look on the arguments for symptom-based treatment on the one and parasite-based treatment on the other hand.

Proponents of the change to general parasite confirmed treatment mainly refer to three points: i) a significant decline of the number of fevers due to malaria in former high transmission areas, ii) the availability of reliable RDTs andiii) an increased likelihood of missing other diseases, when focusing on the diagnosis 'malaria'.

As figures of the last years show, some of the formerly high transmission areas turned into moderate or low transmission areas (Ceesay et al. 2010, Guerra et al. 2008), which consequently results in inappropriate use of anti-malarials in more and more cases, if treatment is presumptive. With declining transmission of malaria the probability of an episode of fever to be due to malaria parasites is also decreasing. In the year 2010 Gething et al. published their comparison of the total number of paediatric fever cases seeking treatment in the public sector in Africa and the number of children really been affected by malaria parasites. According to this spatial analysis 43% of all children seeking medical help were infected with *P.falciparum*, which would have resulted in 104 million cases of over-treatment in the year 2007 if following the presumptive treatment strategy. Albeit, the largest differences between malarial and non-malarial fever cases were found in countries of unstable or low transmission (Gething et al. 2010).

Rapid diagnostic tests have been proven to deliver comparable performance to professional microscopy (Ochola et al. 2006) and use in low standard settings was described to be sufficient (Abeku et al. 2008, Bharti et al. 2008). The diagnostic use of RDTs depends on the prevalence of malaria: In settings with a high prevalence of malarial infections the test will not provide any useful information, as most of the tested patients will be parasite positive and it is not possible to differentiate between symptomatic and asymptomatic parasitaemia nor to exclude other diseases as the cause of fever (Graz et al. 2011). Apart from that the use of RDTs in settings such like this is not cost-effective, particularly not if the costs of the test exceed the costs of the medication (Lubell et al. 2008, Msellem et al. 2009).

Although overall data about test-related action of health workers is rather ambiguous, the over-reliance to positive test results has frequently been described as a major barrier to better results in overall mortality. Especially when treating physicians or health workers focus on malaria as the cause of a fever and refrain other diagnostics (Graz et al. 2011, Willcox et al. 2009). Reyburn et al. even observed higher case

fatality rates in malaria-negative cases than in those who were positive and treated (Reyburn et al. 2004). On the other hand there is also data that indicates an increased use of antibiotics in case of negative test results (Ansah et al. 2010, Msellem et al. 2009).

However, even in the future RDTs for malaria will not be able to exclude other diseases and implementation of additional diagnostic equipment requires money, expertise and time. Meanwhile, it is not much known about the real impact of bacterial bloodstream infections, but the existing figures let hypothesize that they cause a significant proportion of fever cases in SSA (Reddy et al. 2010). As well as typhoid fever, which is a common reason for hospital attendance (Lutterloh et al. 2012, Marks et al. 2010, Neil et al. 2012), bacteraemia and septicaemia of other origin is often accompanied with overlapping symptoms to malaria (Brent et al. 2006). To be able to distinguish between certain conditions the treating health worker or physician must be familiar with the local epidemiology of diseases and their clinical presentations. As in countries where the seasons predefine the incidences for e.g. respiratory tract infections in the dry season and malaria and typhoid fever in the rainy season, distribution of disease differs between regions and countries.

Therefore clinical algorithms as presented in this study can help to give an evident feedback of common clinical presentations and their association to a certain diseases.

Aside from that, health workers indeed play a key role for correct assessment and treatment of children with malaria. They are frequently forced to decide about treatment under great pressure, because often children are presented to them only once, due to access barriers to hospitals such as long travel distances, costs or lack of health insurance coverage (Sarpong et al. 2010).

As experiences with RDTs have shown translation of policies and their changes are not feasible without proper training and evidence based feedback (McMorrow et al. 2008, Reyburn et al. 2007).

For the future improved surveillance systems for disease epidemiology on a regional basis and intense training of health workers based on local experiences would be highly desirable. Continuous reviews of patients could lead to more conservative treatment strategies. Further it is necessary to keep on track with distribution of equipment for malaria and bacterial diseases diagnostics.

4.8 Limitations of this study

The present study has some limitations, which need to be taken into account for interpretation of the results.

Although the overall conditions in the Agogo Presbyterian Hospital were comparable to other settings in West Africa, the APH is involved in medical research for a longer period of time. It is well connected to surrounding villages and the population might be more open-minded for seeking professional medical help instead of visiting traditional healers as they or their relatives might have successfully participated in earlier studies. Another part of the present study revealed an average 'symptom-to-hospital-time' of 3.8 days, which is very short compared to other studies (Gay-Andrieu et al. 2005, Modiano et al. 1998) and could be an expression of good adherence of the population to conventional medicine.

But, consequently, clinical presentations of patients included in this analysis might be also less severe and biased by a better health care seeking behavior in the present study population.

Conditions with overlapping symptoms to malaria have been frequently described. Ideally it should have been possible to examine every child for potential coincidential diseases, but due to non-existing microbiological infrastructure and high costs for equipment this was not feasible and not part of the study. Further on, all signs and symptoms in the analysis were clearly related to and predictive values were calculated for the outcome '*P. falciparum* parasitaemia'. However, comparison with other studies that used 'clinical malaria' as primary endpoint may be difficult.

4.9 Conclusion

The present study revealed a clear age dependency of the predictive value of the symptoms *fever* and *palmar pallor*. The use of the age-derived CART-algorithm increases the specificity of the prediction for *P.falciparum* parasitaemia. The symptom palmar pallor is easy to recognize and might be helpful for health workers as an indicator not only for anaemia but also for malarial parasitaemia. Palmar pallor is able to indicate significant lower Hb-levels even in a population with a high prevalence of anaemia. Due to a lack of sensitivity neither the best algorithm nor *palmar pallor* as a single sign are eligible for decision-making and cannot replace laboratory diagnosis or the use of RDTs. Where these diagnostics are not available all children suspected of malaria have to be treated presumptively.

5. Summary

For the year 2009, the World Health Organization (WHO) estimated 225 million cases of malaria worldwide. Fifty-eight percent of all deaths on the African continent due to malaria were children under 5 years of age.

As malaria incidences are reported to decline over the last years other conditions causing similar symptoms as clinical malaria are gaining in relevance. While laboratory equipment is often missing in resource poor settings presumptive treatment is still common, but the diagnosis of malaria is usually based on the personal experience of the treating health professional. This study aimed to generate an age-derived clinical algorithm with simple signs and symptoms for the diagnosis of *P. falciparum* parasitaemia.

The study was conducted in a rural hospital in the Ashanti Region, Ghana, which is holoendemic for malaria throughout the year. In total, 5447 visits of 3641 patients between 2-60 months of age who attended the outpatient department (OPD) were analyzed. All children were clinically examined by a paediatrician and a full blood count and thick smear were done. A Classification and Regression Tree (CART) model was used to generate a clinical algorithm to predict malarial parasitaemia and predictive values of all symptoms were calculated.

Malarial parasitaemia was detected in children between 2-12 months and between 12-60 months of age with a prevalence of 13.8% and 30.6%, respectively. The CART revealed age-dependent differences in the ability of the variables to predict parasitaemia. While *palmar pallor* was the most important symptom in children between 2-12 months, a *report of fever* and an *elevated body temperature* of ≥ 37.5°C gained in relevance in children between 12-60 months. The variable palmar pallor was significantly (p<0.001) associated with lower haemoglobin levels in children of all ages. Compared to the Integrated Management of Childhood Illness (IMCI) algorithm the CART had higher specificity and positive predictive values for a malarial parasitaemia.

Use of age-derived algorithms can increase the specificity of the prediction for *P.falciparum* parasitaemia. The predictive value of *palmar pallor* is able to indicate significant lower haemoglobin - levels even in a population with a high prevalence of anaemia and should be therefore underlined in health worker training. Due to a lack

of sensitivity neither the best algorithm nor palmar pallor as a sign are eligible for decision-making and cannot replace presumptive treatment of laboratory diagnosis.

Zusammenfassung

Die Weltgesundheitsorganisation WHO schätzte die Zahl der anMalaria erkrankten Menschen im Jahr 2009 auf weltweit 225 Millionen. Auf dem afrikanischen Kontinent betrafen 85% der durch Malaria verursachten Todesfälle Kinder unter fünf Jahren. Obwohl die Inzidenzen der *P.falciparum*-Malaria in einigen Teilen des subsaharischen Afrika sinken und andere Erkrankungen mit ähnlichen Symptomen wie denen der Malaria an Bedeutung gewinnen, ist eine vorsorgliche medikamentöse Behandlung im Verdachtsfall weiterhin üblich. Ziel dieser Arbeit ist die Generierung eines auf das Lebensalter bezogenen klinischen Algorithmus, der mit einfachen klinischen Symptomen die Diagnose einer *P.-falciparum*-Parasitämie ermöglicht.

Die Studie wurde in einem ländlichen Krankenhaus in der Ashanti-Region in Ghana durchgeführt, welche über das ganze Jahr hinweg holoendemisch für Malaria ist. Insgesamt wurden 5447 ambulante Besuche von 3641 Patienten im Alter zwischen 2-60 Monaten analysiert. Alle Kinder wurden von einem Pädiater klinisch untersucht und es wurden ein kleines Blutbild sowie ein Malariaausstrich ('Dicker Tropfen') angefertigt. Mit Hilfe eines*Classification and Regression Tree* (CART) wurde ein klinischer Entscheidungsbaum für die Prädiktion einer Plasmodium-Parasitämie generiert und prädiktive Werte für alle erfassten Symptome berechnet.

Eine Parasitämie wurde bei Kindern im Alter von 2-12 Monaten mit einer Prävalenz von 13.8% und bei Kindern im Alter zwischen 12 und 60 Monatenmit einer Prävalenz von 30.6% gefunden. Das CART-Modell ergab altersabhängige Unterschiede in der Fähigkeit der Variablen eine Parasitämie vorherzusagen. Während sich bei Kindern im Alter zwischen 2 und 12 Monaten die „*palmare Blässe*" als das wichtigste Symptom herausstellte, gewannen die Variablen „*Fieber in der Anamnese*" und „*erhöhte Körpertemperatur ≥ 37.5°C*" bei Kindern im Alter zwischen 12 und 60 Monaten an Bedeutung. Die Variable „*palmare Blässe*" war bei Kindern jedes Alters signifikant ($p<0.001$) mit niedrigeren Hämoglobinwerten assoziiert. Im Vergleich zumAlgorithmus des*Integrated Management of Childhood Illness* (IMCI) hatte das CART-Modell eine deutlich höhere Spezifität sowie einen höheren positiven prädiktiven Wert für die Vorhersage einer Parasitämie.

Schlussfolgerung

Die Anwendung von altersbezogenen Algorithmen erhöht die Spezifität der Vorhersage einer *P.-falciparum*-Parasitämie. Selbst in einer Population mit einer hohen Prävalenz an Anämie ermöglicht der prädiktive Wert der *„palmaren Blässe"* eine Erkennung von signifikant geringeren Hb-Werten. Die Bedeutung der *„palmaren Blässe"* sollte daher in der Schulung von Gesundheitshelfern hervorgehoben werden. Mangels ausreichender Sensitivität kann allerdings weder auf Basis des besten Algorithmus noch mit *„palmarer Blässe"* als einzelnem klinischem Zeichen eine Therapieentscheidung getroffen werden. Sie sind daher kein Ersatz für eine vorsorgliche medikamentöse Behandlung und einen Erregernachweis.

6. Appendix

6.1 Tables

Table A1. **Re-occurence of *palmar pallor* in 1125 patients who had multiple visits (n = 2931) to the outpatient department**

Palmar Pallor on first visit	Pallor on subsequent visit		Total
	No	Yes	
No	2662 (95.8%)	117 (4.2%)	2779 (100%)
Yes	132 (86.8%)	20 (13.2%)	152 (100%)

McNemar-test: chi^2: 0.90 ; degrees of freedom:1 ; Prob>chi^2: 0.343

Table A2. Undernutrition[a] and wasting[b] among children in different ages

	Age in months	Number of patients $\geq$ -2SD[c] (%)	Mean	95% - CI[d]
Undernutrition[a]	2 - 12 (n=1295)	132 (10.19)	-0.41	(-0.48) - (-0.34)
	12 - 24 (n=1412)	168 (11.90)	-0.75	(-0.81) - (-0.69)
	24 - 36 (n=1286)	141 (10.96)	-0.83	(-0.88) - (-0.77)
	36 – 48 (n=807)	84 (10.41)	-0.83	(-0.90) - (-0.76)
	48 – 60 (n=621)	103 (16.59)	-1.08	(-1.15) - (-1.00)
Wasting[b]	2 - 12 (n=1245)	150 (12.05)	-0.44	(-0.53) - (-0.36)
	12 - 24 (n=1375)	175 (12.73)	-0.77	(-0.83) - (-0.71)
	24 - 36 (n=1254)	101 (8.05)	-0.60	(-0.66) - (-0.54)
	36 – 48 (n=783)	41 (5.24)	-0.53	(-0.60) - (-0.46)
	48 – 60 (n=593)	62 (10.46)	-0.80	(-0.89) - (-0.71)

[a] Undernutrition: $\geq$ 2 standard deviations below the median weight-for-age z-score
[b] Wasting: $\geq$ 2 SDs below the median weight-for-height z-score
[c] $\geq$2 standard deviations below the corresponding mean of the certain variable
[d] 95% - Confidence interval

Table A3. Correlation between presence of palmar pallor and lower weight-for-age z-scores[a] in patients of different ages

Age of patients (months)	Weight-for-age z-score[a] (mean)		p-value[b]
	Palmar pallor not present	Palmar pallor present	
2 – 12	-0.38 [CI: (-0.45) – (-0.31)]	-0.82 [CI: (-1.13) – (-0.50)]	0.001
12 – 60	-0.81 [CI: (-0.85) – (-0.78)]	-1.11 [CI: (-1.22) – (-1.00)]	<0.001
2 – 60	-0.71 [CI: (-0.74) – (-0.68)]	-1.06 [CI: (-1.17) – (-0.95)]	<0.001

[a] Weight-for-age z-score was calculated to estimate the grade of undernutrition
[b] The p-value was calculated by assuming a Students distribution and t-testing the hypothesis that there is no difference of the mean weight-for-age z-score between patients with and those without palmar pallor.
Note: CI = 95% - Confidence - interval

6.2 Figures

	n[1]	failure[2]	OR[3]
no → report of fever → no	194	5	.17
yes	435	47	.73
elevated body temperature yes → no respiratory symptoms → no	237	38	1.09
yes	86	28	2.21
palmar pallor yes → no respiratory symptoms → no	54	16	2.01
yes	25	18	4.88
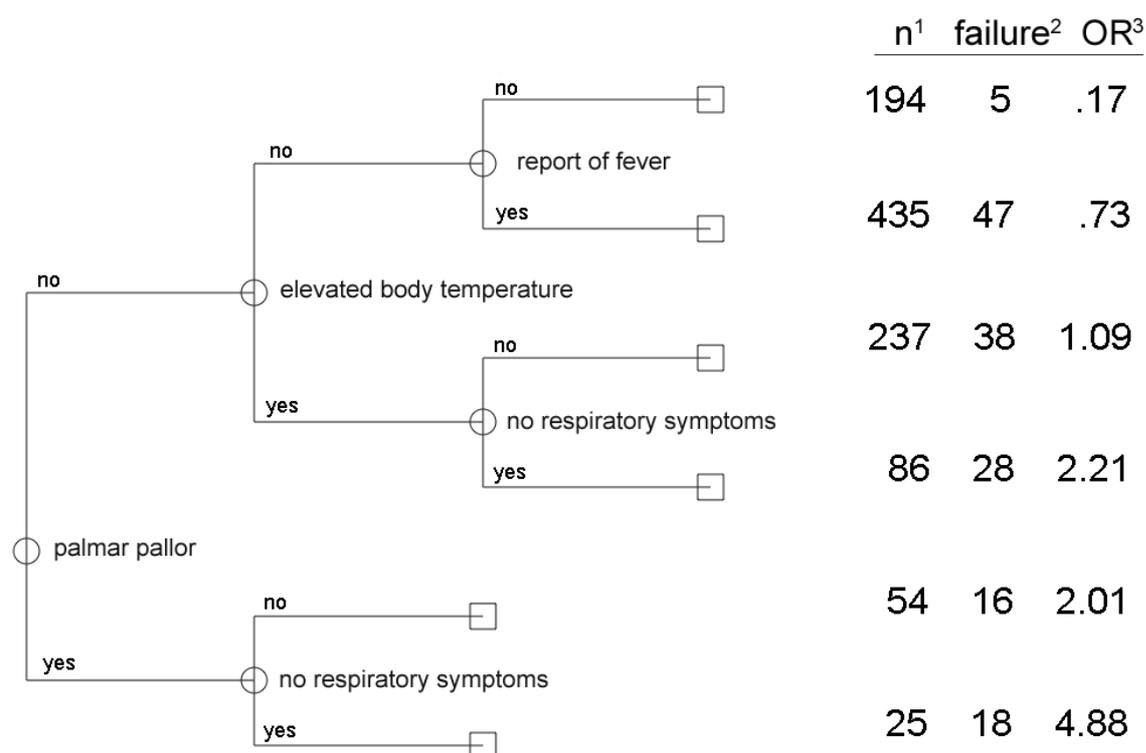
Figure A1. CART for the first visit* of children between 2 and 12 months of age (n=1031)

	n[1]	failure[2]	OR[3]
report of fever — no	352	24	.22
vomiting — no	576	89	.49
vomiting — yes	264	63	.76
palmar pallor — yes	51	20	1.25
palmar pallor — no	237	60	.81
palmar pallor — yes	27	15	1.77
vomiting — yes	112	56	1.60
vomiting — no	362	108	.95
vomiting — yes	194	91	1.50
palmar pallor — yes	99	65	2.10
no respiratory symptoms — yes	336	226	2.15
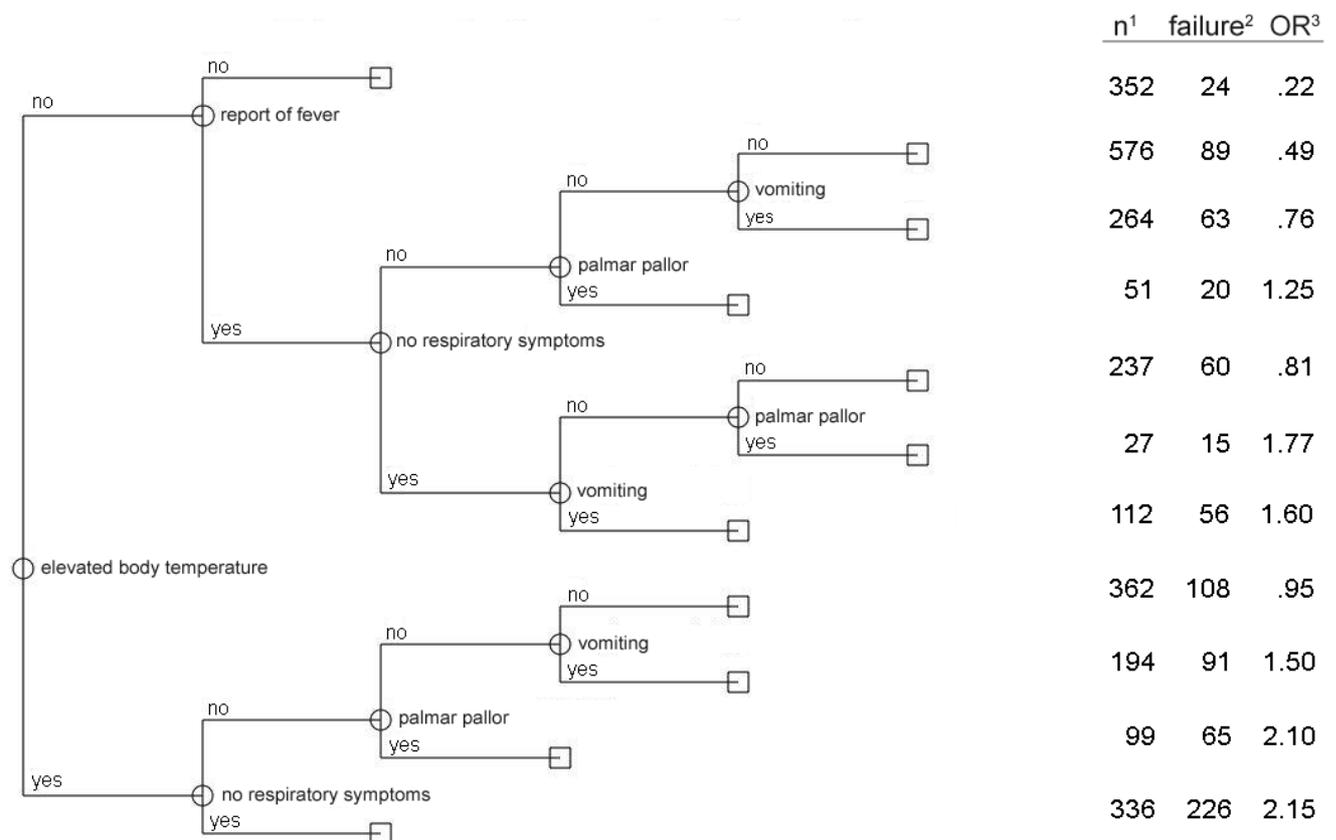
**Figure A2. CART for the first visit* of children between 12 and 60 months
of age (n=2610)**

7. Literature

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Lebenslauf

Ich wurde am 28. September 1980 in Willich geboren. Meiner Einschulung im Juli 1987 in die Gemeinschaftsgrundschule Eichendorfstraße in Kempen schloss sich der Besuch des Gymnasium Thomaeum in Kempen ab Juli 1991 an. Dort beendete ich im Jahr 2000 die gymnasiale Oberstufe mit den Abiturfächern Deutsch, Pädagogik, Mathematik und Wirtschafts- und Sozialwissenschaften.

Im August 2000 begann ich meinen elfmonatigen Zivildienst in der chirurgischen OP-Abteilung des Klinikum Krefeld. In den darauffolgenden Jahren war ich in jeweils zeitlich befristeten vollzeitigen Arbeitsverhältnissen in den OP-Abteilungen der Zentren für Allgemein- und Viszeralchirurgie, Herz- und Thoraxchirurgie sowie der Hals-Nasen-Ohren- und Augenklinik des Klinikum Krefeld bzw. des Helios Klinikum Krefeld (seit 2006) als pflegerische Aushilfe beschäftigt.

Seit dem Beginn meines Studiums der Humanmedizin an der Georg-August-Universität Göttingen im Oktober 2004 habe ich diese Tätigkeit nur noch in den Semesterferien ausgeübt. Darüberhinaus habe ich in den Jahren 2005 bis 2007 als pflegerische Aushilfe auf der internistischen Intensivstation der Medizinischen Kliniken I und II im Klinikum Krefeld gearbeitet.

Den vorklinischen Studienabschnitt beendete ich im April 2006 mit der Ersten Ärztlichen Prüfung. Im klinischen Studienabschnitt absolvierte ich die Wahlpflichtfächer "Tropenkurs für Mediziner", "Advanced clinical anatomy of the internal organs" an der N.U.I. Galway in Irland und "Diagnostik angeborener Herzfehler". Neben dem Studium war ich außerdem von 2006 bis 2009 in der bakteriologischen Abteilung des Labors Wagner&Stibbe in Göttingen beschäftigt.

Im Januar 2009 begann ich in der Abteilung für Infektionsepidemiologie am Bernhard-Nocht-Institut für Tropenmedizin in Hamburg meine Dissertation. Im Rahmen dieser Arbeit war ich von März bis Juli 2009 am Kumasi Centre for Collaborative Research in Tropical Medicine in Kumasi, Ghana sowie am Agogo Presbyterian Hospital in Ghana tätig.

Im Rahmen einer Kooperation der Georg-August-Universität Göttingen mit den Helios Kliniken verbrachte ich im Jahr 2010 ein Praxissemester an zwei Helios Kliniken in Berlin. Mein Praktisches Jahr absolvierte ich in den Kliniken für Gefäßchirurgie, Kardiologie, Hämatologie/Tumorimmunologie sowie Anästhesiologie am Helios Klinikum Berlin-Buch. Die Ausbildung im chirurgischen Teil des

praktischen Jahres erfolgte zudem im Edendale Hospital Pietermaritzburg der University of KwaZulu-Natal in Durban, Südafrika.

Den Zweiten Teil der Ärztlichen Prüfung absolvierte ich November 2011, die Approbation als Arzt erfolgte im Dezember 2011.

Von Februar bis Ende März des Jahres 2012 war ich im Rahmen des "Typhoid Surveillance in Africa Program (TSAP)" für das International Vaccine Institute (Seoul, Südkorea) in Ouagadougou, Burkina Faso, tätig.

Seit Juli 2012 bin ich in der Sektion Infektiologie/Tropenmedizin der I. Medizinischen Klinik am Universitätsklinikum Hamburg-Eppendorf als Assistenzarzt beschäftigt.

Christof Vinnemeier