Exploring the context and potential benefits of implementing an Intermittent Preventive Treatment for malaria in infants (IPTi) in Papua New Guinea

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« Tandis que le sage cherche le pont, le fou traverse la rivière »

Proverbe nord-iranien

Table of Contents

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Summary

Background

Intermittent preventive treatment (IPTi) is an intervention aiming to reduce the risk of malaria in infants. Its concept is to deliver a full treatment course of antimalarial drugs to infants, three or four times during the first year of life, following the expanded program of immunization (EPI) schedule and regardless of clinical malaria episodes.

Mainly Sulphadoxine/pyrimthamine (SP) was studied and demonstrated to reduce the risk of malaria by 30% in Africa, where *Plasmodium falciparum* (*Pf*) is the predominant species. No study has been carried out in regions of the world with a significant burden of non-*Pf* infections. There is therefore a need to investigate the potential benefits of IPTi in areas, such as Papua New Guinea (PNG), highly endemic for *Plasmodium vivax* (*Pv*) malaria.

Apart from efficacy, which is the corner stone of an intervention, it is essential to have a clear picture of the context in which such an intervention might be implemented. Indeed, determinants of effectiveness such as the malaria context (epidemiology and case management), the acceptability and the access to the intervention need to be investigated prior to the implementation of IPTi.

The present study investigates the efficacy of IPTi in PNG as well as some key aspects of infant's health in PNG that might help to understand the context in which IPTi could be implemented.

Methods

Randomized controlled trial investigating the protective efficacy of 4 doses of SP associated to 3 days of artesunate (SP-AS3) or 3 days of amodiaquine (SP-AQ3) given at 3-month intervals during the first year of life. Most of infants were followed-up for an additional 12 months. The study took place in Madang and Maprik (PNG) from 2006 to 2010, but only the Madang cohort was used for the IPTi efficacy analyses.

Making use of the morbidity passive case detection of the trial, the following aspects of infant's health in PNG were investigated:

- Effectiveness of treating infants with a unified treatment (artemther/lumefantrine, AL) for *Pf* & *Pv* malaria based on the result of the rapid diagnostic test (RDT)
- The incidence of common illnesses based on RDT and syndromic definitions of diseases and the impact of IPTi on them.
- The performances of the integrated management childhood illness (IMCI) supplemented with RDT & IPTi for the management of common syndromes/diseases in PNG.

The acceptability of IPTi was also assed alongside the drug trial.

Findings

1605 infants 3 months old were enrolled in the IPTi trial, 1125 in Madang and 480 in Maprik. The intention-to-treat relative risk (RR) at 15 months of age (Madang site only) was 0.72 (95%CI, 0.57 - 0.90) on all malaria episodes with SP-AQ3 and 0.88 (95%CI, 0.70 - 1.10) with SP-AS3, overall p=0.017. Using SP-AQ3, the RR was 0.63 (95%CI, 0.45 - 0.88) on *Pf* and 0.78 (95%CI, 0.60 - 1.01) on *Pv*. No difference was observed in the incidence of overall morbidity, severe diseases and non-malarial illnesses between the placebo and IPTI intervention arms. Fewer deaths were observed in the treatment arms compared to placebo: placebo=8, SP-AQ3=1 and SP-AS3=3.

7223 fever episodes occurred (in Madang and Maprik) during the study and 5670 had a negative RDT result. Out of them, 133 (3.4%) re-attended the clinic within 7 days for fever, and 1 died of lower respiratory tract infection (LRTI). 23 (0.6%) infants re-presented with a severe illness (4 with positive BS and/or RDT). 1728 children with positive RDT results were treated with artemether/lumefantrine (AL). 30 (1.7%) re-attended within 7 days for fever, none died.

Out of the total cohort, incidence rates (episodes/child/year) for common syndromes/diseases were: 0.85 (95%CI, 0.81-0.90) for LRTI, 0.72 (95%CI, 0.65-0.93) for gastroenteritis (GI), 0.62 for malaria (95%CI, 0.58 - 0.66) and 0.08 (95%CI, 0.07-0.09) for otitis.

The introduction of RDT led to a high accuracy of “on site” malaria diagnosis (Κ =0.99). On the opposite, the clinical diagnosis accuracy for others syndromes was poor : Κ=0.47 for LRTI, Κ=0.52 for GI and Κ= 0.52 for otitis.

25% of illness episodes were inappropriately treated: 6% did not receive antibiotics when they should have and 19% received antibiotics when they should not have (according to recommendations). The prescription’s rate of antibiotics was 56% when the RDT for malaria was negative and 16% when the RDT was positive ($p<0.001$). The acceptability of IPTi appears to be good in Melanesian populations.

Conclusion

The use of RDT and artemether/lumefantrine is a safe and effective strategy for the management of malaria cases in PNG and could be implemented very easily. IPTi has demonstrated its efficacy to reduce both *Pf* and *Pv* episodes. However, the apparent absence of benefit on the overall morbidity and on severe illnesses is a concern and mitigates the interest of implementing this intervention in PNG. Furthermore, other factors such as a low EPI coverage and rapidly changing malaria endemicity due to the recent introduction in PNG of insecticide treated nets (ITN) and artemisinin combination therapies (ACT) are likely to jeopardize the potential benefits of IPTi in PNG.

Zusammenfassung

Hintergrund

Intermittent preventive treatment in infants (IPTi) ist eine Intervention mit dem Ziel, das Risiko von Malaria bei Kleinkindern zu reduzieren. Sein Konzept ist den Säuglingen Behandlung mit Malariamedikamenten, drei oder viermal während des ersten Lebensjahres, nach dem Zeitplan des Erweitertens Programms der Immunisierung (EPI) und unabhängig von klinischen Malaria Episoden.

Haupsächlich Sulfadoxin/pyrimthamine (SP) wurde studiert und zeigte, dass das Risiko von Malaria um 30% in Afrika reduziert wurde, wo Plasmodium falciparum (Pf) die vorherrschende Spezies ist. Keine Studie wurde in den Regionen der Welt mit einer signifikanten Belastung durch nicht-Pf-Infektionen durchgeführt. Es besteht daher ein Bedarf, die potenziellen Vorteile der IPTi in Bereichen wie Papua-Neuguinea (PNG), hoch endemisch für Plasmodium vivax (Pv) Malaria, zu untersuchen.

Abgesehen von Wirksamkeit, die der Grundstein für eine Intervention ist, ist es wichtig, ein klares Bild des Kontextes zu haben, in dem ein solcher Eingriff durchgeführt werden könnte. Tatsächlich, Determinante der Wirksamkeit wie der Malariakontext (Epidemiologie und Case Management), die Akzeptanz und der Zugang auf die Intervention, muss vor der Durchführung von IPTi untersucht werden.

Die vorliegende Studie untersucht die Wirksamkeit von IPTi in PNG sowie einige der wichtigsten Aspekte der kindlichen Gesundheit von Kleinkindern in PNG, die helfen könnten, den Kontext, in dem IPTi implementiert werden könnte, zu verstehen.

Methoden

Randomisierte kontrollierte Studie, die die schützende Wirkung untersucht von vier Dosen von SP und 3 Tagen von Artesunat (SP-AS3) oder 3 Tage von Amodiaquin (SP-AQ3) in 3-monatigen Abständen, gegeben während des ersten Lebensyahres. Die meisten Kinder wurden für weitere 12 Monate beobachtet.

Die Studie fand in Madang und Maprik (PNG) von 2006 bis 2010 statt, aber nur die Madang Kohorte wurde für die IPTi Wirksamkeit Analyse verwendet.
Folgende Aspekte der Gesundheit der Kleinkinder in PNG wurden untersucht, indem die passiven Fälle der Studie registriert wurden:

• Wirksamkeit der Behandlung von Säuglingen mit einer einheitlichen Behandlung (artemther / Lumefantrin, AL) für *Pf* & *Pv* Malaria, dem Ergebnis des rapid diagnostic test (RDT) nach.
• Die Inzidenz von gewöhnlichen Krankheiten nach RDT und syndromalen Definitionen von Krankheiten und die Auswirkungen von IPTi auf ihnen.
• Die Leistungen des integrierten Managementsystems Kinderkrankheiten (IMCI) ergänzt mit RDT & IPTi für die Bewirtschaftung der gewöhnlichen Syndrome / in PNG.

Die Akzeptanz von IPTi wurde während dieser Studie bestimmt .

Resultate

1605 3-Monate alt Säuglinge wurden in die IPTi Studie eingezogen, 1125 in Madang und 480 in Maprik. Intention-to-treat relative Risiko (RR) bei 15 Monaten alt betrug 0.72 (95% CI, 0.57 bis 0.90) auf allen Malariaepisoden mit SP-AQ3 und 0.88 (95% CI, 0.70 bis 1.10) mit SP-AS3, Insgesamt p = 0.017. Mit SP-AQ3 wurde das RR 0.63 (95% CI, 0.45 bis 0.88) auf Pf und 0.78 (95% CI, 0.60 bis 1.01) auf Pv. Es wurde kein Unterschied in der Inzidenz von insgesamter Morbidität, schwere Erkrankungen und nicht-Malaria-Erkrankungen zwischen der Placebo-und IPTi Intervention Arme beobachtet. Nur wenige Todesfälle wurden in den Behandlungsgruppen im Vergleich zu Placebo beobachtet: Placebo = 8, SP-AQ3 = 1 und SP-AS3 = 3 ist.

7223 Fieberepisode (in Madang und Maprik) sind während der Studie aufgetreten und 5670 hatten einen negativen RDT. Aus ihnen, 133 (3.4%) besuchten die Klinik innerhalb von 7 Tagen bei Fieber wieder und 1 starb an unteren Atemwegeinfektion (LRTI). 23 (0.6%) Säuglinge kamen erneut mit einer schweren Krankheit (4 mit positiven BS und / oder RDT). 1728 Kinder mit

positiven RDT Ergebnissen wurden mit Artemether / Lumefantrin (AL) behandelt. 30 (1.7%) besuchten die klinik innerhalb von 7 Tagen bei Fieber wieder, keiner starb.

Von der insgesamten Kohorte waren Inzidenzraten (Episode / Kind / Jahr) für gemeinsame Syndrome / Krankenheiten : 0.85 (95% CI, 0.81-0.90) für LRTI, 0.72 (95% CI, 0.65-0.93) für Gastroenteritis (GI), 0.62 f!ur malaria (95%CI, 0.58 - 0.66) und 0.08 (95% CI, 0.07-0.09) für Otitis.

Die Einführung von RDT führte zu einer hohen Genauigkeit von "on site" Malaria-Diagnose (Κ = 0.99). Im gegenteil, war die Genauigkeit klinischer Diagnose für andere Syndrome schlecht □: Κ = 0.47 für LRTI, Κ = 0.52 für GI und Κ = 0.52 für Otitis.

25% der Krankheitsepisoden wurden unsachgemäß behandelt: 6% erhielten keine Antibiotica, wenn sie sie haben sollten und 19% erhielten Antibiotika, wenn sie sie nicht haben sollten (den Empfehlungen nach). Die Verschreibung von Antibiotika war 56% wenn der RDT für Malaria negative war und 16% wenn der RDT postiv war (p <0.001). Die Akzeptanz für IPTi scheint in melanesischen Bevölkerung gut zu sein.

Schlussvolgernd

Die Verwendung von RDT und Artemether / Lumefantrin ist eine sichere und wirksame Strategie für die Verwaltung der Malariafälle in PNG und könnte sehr leicht realisiert werden. IPTi hat seine Wirksamkeit demonstriet, sowohl Pf und Pv Episoden zu reduzieren. Allerdings ist die offensichtliche Abwesenheit von Vorteilen auf die gesamte Morbidität und auf schwere Krankheiten ein Anliegen, und mildert das Interesse der Durchführung dieser Intervention in PNG. Darüber hinaus gibt es andere Faktoren, wie eine niedrige EPI Berichterstattung und die sich rasch verändernde Malaria endemizität durch die kürzliche Einführung in PNG von Insektiziden behandelte Moskitonetze (ITN) und Artemisinin-Kombinationstherapien (ACT), die die potenziellen Vorteile der IPTi in PNG gefährden könnten.

List of abbreviations

ACT	Artemisinin combination therapies
AL	Artemether-lumefantrine
AOM	Acute otitis media
AQ	Amodiaquine
ARI	Acute respiratory tract infeciton
AS	artesunate
BS	Blood slide
CL	Chloroquine
DALY	Disability-adjusted life year
DP	Dihydroartemisinin - piperaquine
DSMB	Data and safety monitoring board
EIR	Entomological inoculation rate
EPI	Expanded program of immunization
GCP	Good clinical practice
Hb	Hemoglobin
Hib	*Haemophilus influenza*
HRP2	Histidine-rich protein-2
IMCI	Integrated management of childhood illness
IPT	Intermittent preventive treatment
IPTi	Intermittent preventive treatment in infants
IPTp	Intermittent preventive treatment in pregnant women
IRR	Incidence rate ratio
IRS	Indoor residual spraying
ITN	Insecticide treated nets
LLIN	Long-lasting insecticide treated nets
LRTI	Lower respiratory tract infection
MCH	Mother and child health clinic
NGO	Non-governmental organization
OPD	Outpatient departement
PE	Protective efficacy
Pf	Plasmodium falciparum
pLDH	parasite-specific lactate dehydrogenase
Pm	Plasmodium malariae
PNG	Papua New Guinea
Po	Plasmodium ovale
Pv	Plasmodium vivax
RCT	Randomized controlled trial
RDT	Rapid diagnostic test for malaria
RR	Relative risk
RSV	Respiratory syncithyal virus

SP	Sulphadoxyne-pyrimethamine
URTI	Upper respiratory tract infection
WHO	World Health organization

List of figures

List of tables

List of Graphs

1 Introduction

1.1 Epidemiology, diagnosis and treatment of malaria

1.1.1 Epidemiology of malaria world wide

Malaria is considered to be one of the major contributors to the burden of diseases in tropical countries. About 236 millions cases of malaria and 781'000 deaths were reported in 2009 according to the World Malaria report of WHO (WHO 2010). Theses figures are approximations based on presumptive diagnosis of malaria, meaning that most cases of malaria reported are not confirmed by the presence of circulating parasites, but only assumed because of the presence of fever or history fever. This approach towards the estimation of the malaria burden creates some problems for public health authorities. Indeed, one of the challenges when implementing control strategies for malaria is to measure accurately the true burden of the disease. This will be extensively discussed in **chapter 3**, but as an example, we can observe that in the world malaria report (WHO 2009) 236 millions of malaria cases are reported which are in fact suspected cases (based on presumptive diagnosis) and only 82 millions (one third) are confirmed cases by microscopy or rapid diagnostic tests (RDT). At a country level, this difference can have important implications for planning control interventions.

1.1.2 Epidemiology of malaria in Papua New Guinea (PNG)

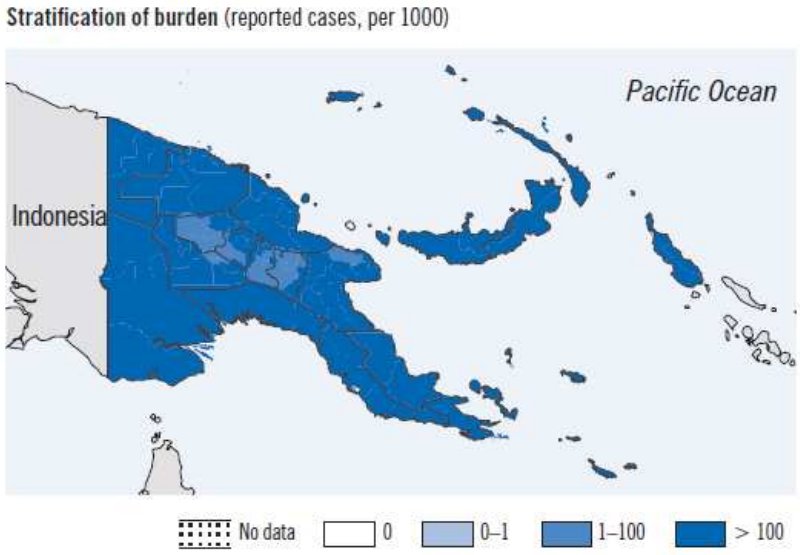
In 2009, 1.6 millions of febrile episodes were reported as suspected malaria cases in PNG. Only 250'000 had a blood examination and one third were found positive(WHO 2009). A survey performed in 2008 in Madang Province (at sea level), found out that approximately half of the patients (general population) with fever visiting an health facility had a positive RDT and/or blood slide (BS) for malaria (Senn, Luang-Suarkia et al. 2011)

Due to its geography and climate, PNG is presenting a wide range of malaria transmission intensities (See **figure 1.1**). It is highly prevalent at sea level, where

rainfalls can be very abundant (up to 7000mm/year). Parasite prevalence rate as high as 50% is reported in asymptomatic school children on the North coast of Madang. (Michon, Cole-Tobian et al. 2007) On the opposite, in the Highlands (above 1500m), malaria transmission is less stable, but is generally much lower as altitude increases. It is however frequent to observe localized and often severe epidemics. For example, in Simbu Province the prevalence rate of malaria parasites was around 5% and climbed up to 13-36% during epidemics.(Mueller, Kundi et al. 2004) Important variations can also occur in low land areas, the South part of the country (much dryer) and some Islands having a lower endemicity.(Mueller, Bockarie et al. 2003) Other factors such as distance to health facilities have also been described to significantly change the distribution of malaria: the longer the distance is the higher the prevalence of malaria is. (Myers, Myers et al. 2009) In past surveys, the entomological inoculation rate (EIR) varied between 1-400 (Mueller, Bockarie et al. 2003).

Transmission of malaria is perennial on costal areas with limited variations across seasons. (Mueller, Bockarie et al. 2003) All four species of plasmodium are present in PNG. *Plasmodium falciparum* (*Pf*) and *Plasmodium vivax (Pv)* are more or less equally prevalent in PNG. (Mueller, Kundi et al. 2004) However, this ratio can vary from one place to another, sometimes in favor of *Pf* and sometimes in favor of *Pv*. The age pattern of *Pf* and *Pv* clinical infections are different, *Pv* being more frequently associated with diseases in younger age.(Lin, Kiniboro et al. 2010) Mixed infections with two or more species are also frequently reported. (Mehlotra, Lorry et al. 2000)

Figure 1-1: Geographical distribution of the reported number of suspected malaria cases per 1000 persons for the year 2008 in PNG (WHO 2009)



1.1.3 Diagnosis of malaria in routine practice

For very long, presumptive diagnosis was used to guide treatment in endemic areas. This means that patients visiting health facilities with fever or history of fever were treated with antimalarials. This strategy, advocated by WHO for many years, is no longer acceptable because of the lack of specificity of fever to make the diagnosis of malaria (D'Acremont, Lengeler et al. 2009). Indeed, this has led to the rapid increase of resistance of parasites against antimalarial drugs, the potential for patients to experience unnecessary side effects and to delay proper management of other causes of fever. Therefore, new recommendations include the need to confirm a malaria infection by either microscopy or RDT. (WHO 2010) If the reading of blood smears (thick and thin films) is still considered by some experts to be the gold standard to confirm clinical malaria it has also limitations in routine practice. Indeed, it requires highly skilled microscopists, important resources and takes a lot of time.

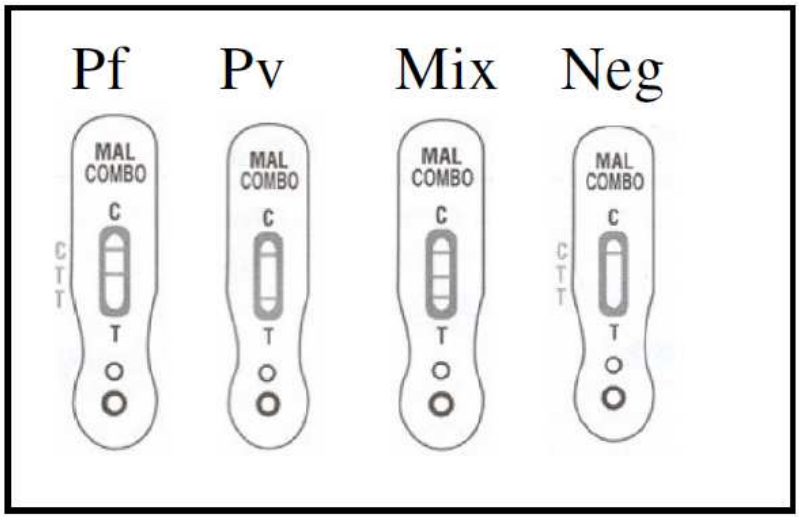
Nowadays, there is growing evidence that RDT could be the best option for an accurate and easy to perform diagnosis of malaria in routine practice. (D'Acremont, Lengeler et al. 2009) All RDT for malaria are working on the same principle. They are based on the detection of circulating antigens of malaria parasites. Usually, an antigen common to all malaria species, the parasite-specific lactate dehydrogenase (pLDH) and an antigen specific for *Pf* identification, histidine-rich protein-2 (HRP2) are used. Depending on the local epidemiological context, only one of the antigens will be used. For example, in Africa, mainly RDT detecting HRP2 are used as *Pf* is the predominant species. On the other hand, in regions such as PNG where both *Pv* and *Pf* species are present, a test combining both antigens need to be used. Identification of antigens is based on antibodies - antigens reactions. After adding a buffer to a few µl of blood, liquid will migrate on filter paper where specific antibodies are bound to the strip and will react in case of presence of antigens. One or more color lines will then appear on the strip of test allowing the health worker to confirm or not a malarial diagnosis. Performances of RDT have been investigated by WHO(WHO 2008) and the usual accepted threshold for parasites detection is 100 parasites/µl. It is however clear, that detecting antigens is not the same thing as detecting whole parasites, therefore RDT and microcpy should not be seen as looking exactly at the same thing and should not be directly compared in terms of performances, especially when assessing patients that were recently treated. All these procedures are explained in details in a document published by WHO. (WHO 2006)

1.1.4 Diagnosis of malaria in PNG

In PNG, until recently, most health facilities were still using presumptive diagnosis for malaria. Only reference hospitals could usually perform a microscopy examination of blood films. A national program was launched in 2010 and aim to implement RDT-based diagnosis in all health facilities.

In the present study, we used a RDT able to detect both antigens for all species and specific for *Pf* (ICT combo®, Cape Town, South Africa)**. Figure 1.2** shows a picture of the test used.

Figure 1-2: ICT Combo® malaria RDT with it interpretation



If the performances of these tests are well established in African countries where *Pf* is the predominant species, their effectiveness in children under 5 years in regions of the world with a high burden of non-*Pf* infections remain unknown. Indeed, these tests are known to have a lower sensitivity for *Pv.* This important issue will be addressed in **chapter 4**.

1.1.5 Treatment of malaria

One of the main achievements for malaria treatment in the past 10 years is the successful introduction of highly efficacious artemininin combination therapies (ACT). Indeed, the “rediscovery” of artemisinin, an old Chinese drug, and its derivates (artemether, artesunate,…) has significantly changed the face of malaria treatment. This highly effective, well tolerated and short acting drug has been introduced in many African countries as first line treatment in combination

with a long acting drug. One of the most well known combinations is artemether-lumefantrine (AL, Coartem® manufactured by Novartis, Switzerland).

1.1.6 Treatment of malaria in PNG

Unlike Africa, PNG did not change its malaria treatment policy until 2010. Indeed, first line treatment for children was a combination of a single dose sulphadoxyne-pyrimethamine (SP) and 3 days amodiaquine (AQ) or chloroquine (CL). Second line treatment was SP associated to 3 days of artesunate (AS). Patients with severe malaria received im artemether with oral SP or quinine in second line. Resistance levels against SP, AQ and CL are very high in PNG.(Marfurt, de Monbrison et al. 2008) Reasons for not having changed policies are numerous, but the most important was the lack of scientific evidence on the efficacy of the new ACT on non-*Pf* malaria. (Douglas, NM. et al. 2010) Only very limited highly controlled trials have specifically looked at their efficacy against *Pv* and concluded that efficacy might be sub-optimal. (Karunajeewa, Mueller et al. 2008) Effectiveness of AL in PNG will be discussed in **chapter 4**. More generally, it is of interest to clarify if AL could be used as a unified therapy, meaning that it is able to treat all malaria species, which might be relevant in regions of the world highly endemic for *Pf* and *Pv* such as PNG. AL was the drug used to treat malaria episodes in children enrolled in the IPTi trial (see below).

1.1.7 Malaria control strategies

Malaria control programs that were implemented in the last decade in most endemic countries were successful and contributed to dramatically decrease the endemicity of malaria worldwide. (Feachem, Phillips et al. 2010) Beside the important increases of financial resources and a better political commitment, interventions such insecticide treated nets (ITN), (Lengeler 2004) indoor residual spraying (IRS), (Pluess, Tanser et al. 2010) accurate diagnosis based on rapid diagnostic tests (RDT) and efficient treatment based on artemisinin combination therapies (ACT) have demonstrated to be efficient tools to decrease the burden of malaria. (Ansah, Narh-Bana et al. 2010; d'Acremont, Malila et al. 2010 ;

Uzochukwu, Onwujekwe et al. 2011) Due to these efficient strategies, almost half of the endemic countries (42 out of 98) have reported a decrease of 50% or more over the past 10 years. (WHO 2010)

1.1.8 Malaria control strategies in PNG

Unlike many countries, Papua New Guinea is still facing high malaria endemicity in most parts of the country and no reduction was observed in the past decade. This is mainly due to delays in implementing control strategies. Indeed, ITN were only introduced in the country in 2009, IRS is not yet available and ACT-based treatments are only going to be implemented in 2011. This is especially preoccupying as most of the surrounding countries such as Indonesia and Salomon Islands are on the way towards malaria elimination through an international initiative called the Asia Pacific Malaria Elimination Network.(APMEN)

1.2 Expanded program of immunization (EPI) and mother & child health clinics (MCH)

Most developing countries have implemented their own immunization schedules, known as expanded program of immunization (EPI) as per the recommendations of WHO. The aim is to immunize infants against most common vaccine-preventable diseases according to a standard schedule. It is usually performed during ad hoc health care visits, or alongside mother and child health clinics (MCH). Small differences between countries may exist both for the type of vaccines provided and the time of immunization. The PNG EPI schedule is shown in **table 1.1**.

In PNG, immunization is provided during MCH clinics (“bebi klinik”), mainly along monthly outreach clinics in villages. Indeed, most of the PNG population is widespread in the countryside; therefore, the only way to achieve reasonable access to vaccine is for health workers to visit the children in their place of

residency instead of making the parents to come to the local health centers. This is not without creating organizational challenges, which is reflected by a low immunization coverage. For example, in 2009, the reported vaccine coverage for the first dose of measles was lower than 60%(WHO 2009). **Figure 1.3** shows a typical scene of one of those clinics.

Table 1-1: Immunization schedule in PNG (Hib vaccine was introduced in 2007)

	Age (months)						
	0	1	2	3	6	9	12
BCG	X						
Oral polio vaccine (OPV)	X	X	X	X			
Hepatitis B (HBV)	X	X		X			
Diphtheria - pertussis - tetanus (DPT)		X	X	X			
Haemophilus influenza (Hib)		X	X	X			
Measles					X	X	
Vitamin A					X		X

Figure 1-3: EPI / MCH clinic in Basken village, North Coast of Madang, Papua New Guinea

From a practical point of view, each child in PNG receives at birth a health book (helt buk in tok pisin) where all attendances to health facilities are recorded

(**figure 1.4**). This includes the vaccine status and a brief summary of each medical visit performed (history, diagnosis and treatment)

<u>Figure 1-4:</u> Nurses checking health books of sick children during outreach MCH clinics & a cover page of a health book

1.3 General concept of IPTi

Several preventive interventions have proven to be efficient in reducing to risk of malaria. Apart from ITN and IRS, intermittent preventive treatments (IPT) have demonstrated some benefits, in particular in pregnant women (IPTp). (Rogerson, Chaluluka et al. 2000) The overall concept is to deliver a full treatment of antimalarial drugs to a given population at fix intervals independently of the malaria status. The concept of intermittent preventive treatment in infants (IPTi) was developed in the late 90'. The aim is to use EPI facilities to deliver antimalarial treatments to infants at the same time as they come for the immunization during the first year of life. WHO is recommending IPTp in all endemic areas and IPTi in some regions with high a endemiciy of malaria (WHO 2010).

Several randomized control trials have investigated IPTi in different African countries with various drug regimens. Sulfadoxine-pyrimethamine (SP) dispensed as a single dose three or four times during the first year of life is the

most studied drug and has shown to have a protective efficacy against clinical malaria episodes of 30% and 21% against anemia (Aponte, Schellenberg et al. 2009). IPTi with SP is still effective in areas with moderate resistance (Mayor 2008; Griffin, Cairns et al. 2010), however, when resistance raises, SP appears to fail to prevent malaria (Gesase, Gosling et al. 2009; Gosling, Gesase et al. 2009). Two clinical trials have investigated other drug regimens including mefloquine (125 mg single dose), a combination of single dose of SP and 3 days of artesunate (AS) or amodiaquine associated to 3 days of AS. All these treatments have similar efficacies compare to SP used alone, ranging from 26 to 38% (Gosling, Gesase et al. 2009; Odhiambo, Hamel et al. 2010). While mefloquine showed the highest efficacy, its tolerability was poor. Shorter acting drug such as dapsone/chlorproguanil used alone did not show any efficacy against malaria (Gosling, Gesase et al. 2009; Odhiambo, Hamel et al. 2010). This is probably due to the fact that IPTi works mainly through a prophylactic effect achieved by long acting drugs (prevention of new infections) rather than through a therapeutic effect (cure of existing parastiemia). (May, Adjei et al. 2008) **Table 1.2** is summarizing the characteristics and main outcomes of the different trials.

All IPTi studies have been exclusively carried out in Africa where *Pf* is the predominant parasite. No studies have been done in regions also highly endemic for *Pv*, which is a major source of morbidities in young children. African results cannot be easily extrapolated to these regions, because of the ability of *Pv* to relapse from dormant forms in the liver and to quickly acquired resistance to SP (Tjitra, Anstey et al. 2008).

Table 1.2: Main characteristics and outcomes of the height IPTi trials carried out in Africa

Investigators	place	Endemicity of malaria (incidence)	drugs regimen*	number of doses	morbidity & follow-up**	Iron suppl.	treatment for acute malaria	Protective efficacy malaria	Protective efficacy anemia (<8 g/dl)	Protective efficacy hospit.	Protective efficacy on outpatient visits
Schellenberg	Tanzania	0.43	SP (stat)	2M-3M-9M	Passive + 2 CS	Yes regular	SP + quinine	59%	50%	30%	no
Chandramohan	Ghana	1	SP (stat)	3M-4M-9M-12M	Passive + 4 CS	Yes regular	CQ + SP or quinine	25%	no	35% (anemia)	only 1 month after dose 1(OR=0.8) & 3 (OR=0.71)
Macete	Mozambique	0.55	SP (stat)	3M-4M-9M	Passive + 2CS	none	Quinine	22%	70-81% 1 month after dose 1 &2	19%	only 1 month after dose 1 for chest indrawing (RR=0.57)
Kobbe	Ghana	1.29	SP (stat)	3M-9M-15M	Passive + CS monthly	none	Art + AQ	20% (After first 2 doses only)	30% (after dose 1 only)	no	no
Grobusch	Gabon	0.22	SP (stat)	3M-9M-15M	Passive + CS monthly	none	AS5 then AS-AQ	no (17% no sign)	no (22% no sign)	no	n/a
Mockenhaupt	Ghana	hyperendemic	SP (stat)	3M-9M-15M	Passive + CS 3-monthly	if Hb low	AS 5d	23%	24%	33% (1 month after dose 1)	no
Gossling	Tanzania (2 sites)	moderate and low	MEF (stat) SP (stat) CD (stat)	2M-3M-9M	Passive + 5 CS	if Hb low	AQ then Art + AQ then Coartem	38% (MEF)	no	no	n/a
Odhiambo	Kenya	app. 1.0 (seasonal)	SP (stat) AQ- AS3 CD3	2M-3M-9M	Passive	Yes regular	Quinine then Coartem	26% (SP-AS) 26% (AQ-AS)	no	no	no

*** SP (stat): sulphadoxyne-pyrimethamine single dose, AS: artesunate, MEF=mefloquine, CD: chlorproguanil - dapsone, AQ: amodiaquine, stat=single dose**

**** CS: cross-sectional surveys (active case detection)**

1.4 Burden of diseases and integrated management of childhood illness (IMCI) in Papua New Guinea

In children under five years, pneumonia, malaria and diarrhoea are the leading causes of death worldwide with an estimate of 5 millions of deaths yearly. More than 90% of them occurred in Africa and countries with limited resources(Bryce, Boschi-Pinto et al. 2005). Morbidity reveals similar pattern with acute respiratory infections, diarrhea and malaria being responsible for the majority of the burden of diseases in developing countries. (Velema, Alihonou et al. 1991; Roca, Quinto et al. 2006; Deressa, Ali et al. 2007; Animut, Mekonnen et al. 2009; Feikin, Olack et al. 2011). In PNG, very limited data are available on the burden of diseases apart from those collected through sporadic local surveys. Indeed, except for malaria, rotavirus and vaccine-preventable diseases, no regular surveillance system exists in the country. (WHO 2009)

Case management of sick children under five in developing countries is usually based on syndromes (presence/absence of signs and symptoms) rather than on etiologies. This strategy has been recommended by the World Health Organization (WHO) who developed the Integrated Management of Childhood Illness (IMCI)(Gove 1997) guidelines for this purpose. Most of the developing countries have adapted these strategies. PNG makes no exception and has adopted the IMCI guidelines. The only changes made compare to the original from WHO are modifications made to stick to the local epidemiology of certain diseases. For example, a special section for Pigbel (a severe gastro-intestinal disease) has been added to the guidelines, including immunization and special care recommendations.

Few studies have looked at the performances of IMCI and potential ways to improve it in PNG. (Moti and Vince 2008 Sep-Dec) This will be the aim of **chapter 6**, where in-depth investigations of the appropriateness of IMCI as well as the potential benefits of adding RDT for malaria and IPTi to IMCI will be discussed.

2 Goal, objectives and methods

2.1 Goal

Exploring the context of children's health in Papua New Guinea and the potential benefits of implementing an Intermittent Preventive Treatment for malaria in infants (IPTi)

2.2 Objectives

Objective 1

Assessing population hemoglobin means and anemia prevalence used as metrics for defining malaria endemicity

Objective 2

Assessing the effectiveness of treating young children with a unified treatment of artemether-lumefantrine based on RDT result in PNG, a highly endemic country for Plasmodium falciparum and vivax

Objective 3

Investigating the efficacy of IPTi with Sulphadoxine/Pyrimethamine associated to either amodiaquine or artesunate on malaria-related morbidity and anemia in Papua New Guinea (all species)

Objective 4

Investigating the potential benefits on diseases management of updating IMCI with rapid diagnostic test and intermittent preventive treatment for malaria in PNG

Objective 5

Investigating the community response (acceptability) to intermittent preventive treatment of malaria in infants (IPTi) in PNG

2.3 Methods

Preliminary comment

Except for chapter 3, all data used for the present work were collected exclusively alongside a randomized controlled trial (RCT) carried out in PNG. Therefore the next two sections (2.3.1 and 2.3.2) refer to this trial.

2.3.1 Brief outline of the design of each of the objective

Objective 1

Convenience sample, multisite cross-sectional household surveys. Correlations (r^2) between population Hb mean and anemia prevalence and altitude, parasite rate and spleen rate were investigated in children age 2-10 years and in the general population

Objective 2

Longitudinal prospective study assessing rates of re-attendance and occurrence of serious adverse events according to RDT results and treatment with AL if positive

Objective 3

Individually randomized, double blind, placebo-controlled trial of two different regimes [sulfadoxine/pyrimethamine (stat) with either 3 days of amodiaquine (SP-AQ3) or 3 days of artesunate (SP-AS3)] of IPTI

Objective 4

Longitudinal study assessing the performances of IMCI+ by looking at the accuracy of diagnosis, the rates of antibiotics' prescriptions, the re-attendance rates according to the initial diagnosis and treatment and the efficacy of IPTi on the overall morbidity and main syndromes

Objective 5

Qualitative assessment of the acceptability of IPTi by administrating questionnaires to mothers whose infants participated in the randomised placebo controlled trial of IPTi. Mothers whose infants participated and who refused to participate in the trial, health workers, community reporters and opinion leaders were interviewed. Men and women from the local community also participated in focus group discussions.

2.4 IPTi study design and procedures

Between 2006 and 2010 a RCT on intermittent preventive treatment for malaria in infants (IPTi) was carried out in two provinces of PNG: Madang and Maprik. It was a double blind, placebo-controlled trial of two different drugs regimes: sulfadoxine/pyrimethamine (stat) with either 3 days of amodiaquine (SP-AQ3) or 3 days of artesunate (SP-AS3). All drugs were given at EPI time points at 3, 6, 9 & 12 months (see **table 1.1** for vaccines schedule, chapter 1). The efficacy of the intervention was assessed at 15 months of age. Afterwards children continued to be monitored every 3 months for up 12 additional months (see study profile, **table 3**)

The 40 study villages are serviced by 4 major health centres (Mugil & Alexishafen in Madang and Kaugia & Kunjingini in the Sepik) and several aid posts. All health centers are responsible to deliver the EPI program in surrounding villages through monthly outreach clinics.

Infants could be enrolled in the trial if the met the following inclusion criteria:

1) Permanent resident of the area
2) Aged 2-4 months old
3) No disability
4) No chronic illness
5) No allergy to study drugs
6) Hb>5g/l
7) Weight for age > 60%.

Children were recruited into the IPTi study alongside monthly outreach MCH clinics by specifically trained study nurses jointly with medical staff of both health centres under the supervision of two study physicians. Regular meetings were carried out to train study nurses to high quality standards for both the study procedures and health care management of sick study participants (**figure 2.1**).

Figure 2-1: Staff training session in Megiar, North Coast of Madang.

The main activities of the IPTi trial are summarized in **table 2.1** (see also chapter 5).

Table 2.1: Summary of visits performed along the IPTi study

Type of Visit	Age (months)									
	2	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**
Pre-screening										
IPTi treatment		IPTi 1	IPTi 2	IPTi 3	IPTi 4					
Primary efficacy assessment										
Follow-up										
Final assessment										
Passive case detection										
Immunization	OPV3	OPV4								
		HBV3								
	DPT2	DPT3								
		Meas 1		Meas 2						
			Vit A		Vit A					
During schedulded visits										
blood collection from finger prick		500µl	250µl	250µl	1 ml	250µl	250µl	250µl	250µl	1ml
Hb level & spleen measurment										

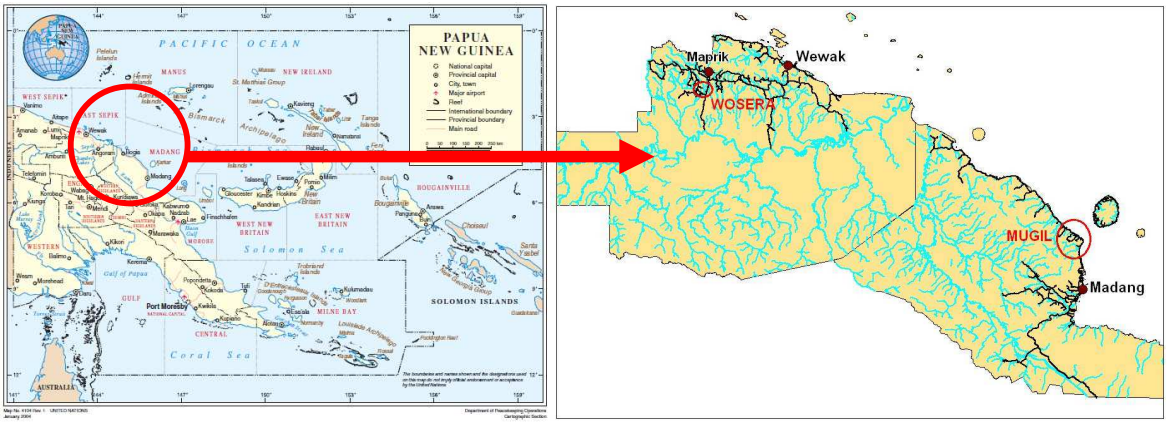
Throughout the study period, a passive case detection system was maintained at all health facilities where all study participants were reviewed when they were sick. In case of history of fever, axillary temperature > 37.5° or signs/symptoms of anemia, the children had a finger prick performed for malaria (RDT) and anemia diagnosis. Apart from malaria and anemia, sick study participants were treated according to IMCI.
See **chapter 5** and **appendix 3** for details on the procedures of the trial.

2.5 Study area and population

2.5.1 Study area

Papua New Guinea occupies the eastern part of the tropical island of New Guinea (which is shared with the Indonesian's territory of Irian Jaya) as well as numerous smaller islands and atolls in the Pacific, for an area of more than 460'000 sq km (**figure 2.2**). The central part of the island rises into a wide ridge of mountains known as the Highlands (Higher summit: Mt Wilhelm, 4509 meters). The coastlines of these islands are volcanic, with steep mountain ranges. The climate is tropical with constant temperature all year around (24°C – 37°C) and abundant rainfalls during the rainy season (October to June).
Most of the project took place in the coastal and lowland provinces of Madang and East Sepik (approximately 500'000 people). Some data used for investigating the relationship between malaria and anemia (**chapter 3**) were collected throughout the Highlands.

Figure 2-2: Map of PNG with study area



2.5.2 Study population

About 6.5 millions people are leaving in PNG. The cultural diversity is very broad with more than 800 different languages and as many different communities. Most of the population is leaving in rural areas (about 85%) and the literacy rate, with 56%, is one of the lowest in the Pacific region. Life expectancy at birth is 53 years, the fertility rate is 4.6 births/women and the population growth is 2.7%. The study population is exclusively situated in rural areas. Most of the participants are leaving in two well delimited regions: Sumkar district (Madang Province) and Maprik district (East Sepik Province). It is estimated that a population of about 40'000 people are leaving in these two areas.

2.5.3 Ethics

The study was carried out in accordance with Good Clinical Practice (GCP) guidelines as required by the International Conference on Harmonization (ICH) GCP E6 and monitored by an independent external monitor. A data and safety monitoring board (DSMB) composed of 2 clinicians and one statistician was responsible to monitor the safety of the study. The protocol was approved by the PNG Medical Research Advisory Committee (MRAC number 05.20). The trial was registered on www.clinicaltrials.gov (number NCT00285662) and formed part of the IPTi consortium (www.ipti-malaria.org).

Parents of study participants had to sign a written consent. In case of illiteracy, a witness was asked to sign. Systematically, a picture-based information notice was shown and given to parents (See **Appendix 1**)

3 Population hemoglobin means and Anemia Prevalence: new metrics for defining malaria endemicity?

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3.1 Abstract

Background

The hypothesis is that hemoglobin-based metrics are useful tools for estimating malaria endemicity and for monitoring malaria control strategies. The aim of this study is to compare population hemoglobin mean and anemia prevalence to established indicators of malaria endemicity, including parasite rates, rates of enlarged spleens in children and records of (presumptive) malaria diagnosis among populations living with different levels of malaria transmission.

Methodology / Principal Findings

Convenience sample**,** multisite cross-sectional household surveys conducted in Papua New Guinea (PNG). Correlations (r^2) between population Hb mean and anemia prevalence and altitude, parasite rate and spleen rate were investigated in children age 2-10 years and in the general population. 21,664 individuals from 156 different communities were surveyed. Altitude ranged from 5 to 2120m. In young children, correlations between altitude and parasite rate, population Hb mean, anemia prevalence and spleen rate were high (r^2: -0.77, 0.73, -0.81 and -0.68; $p<0.001$). In the general population, correlations between altitude and population Hb mean and anemia prevalence were 0.83 and 0.85, respectively. Among young children, parasite rate correlated highly with anemia prevalence, population Hb mean and spleen rate (r^2: 0.81, -0.81 and 0.86; $p<0.001$). Population Hb mean (corrected for direct altitude effects) increased with altitude, from 10.5 g/dl at <500 m to 12.8 g/dl at >1500 m ($p<0.001$)

Conclusions

In PNG, where Plasmodium vivax accounts for an important part of all malaria infections, population hemoglobin mean and anemia prevalence correlate well with altitude, parasite and spleen rates. Hb measurement is simple and affordable, and may be a useful new tool, alone or in association with other metrics, for estimating malaria endemicity and monitoring effectiveness of

malaria control programs. Further prospective studies in areas with different malaria epidemiology and different factors contributing to the burden of anemia are warranted to investigate the usefulness of Hb metrics in monitoring malaria transmission intensity.

3.2 Introduction

Malaria, with about 500 million cases reported per year worldwide, remains one of the predominant diseases in tropical countries. The intensity of malaria transmission depends on a variety of factors such as climate and geographical context, human population, socio-economical level and vector ecology, as well as the type of control strategies in place. Nowadays, several efficient strategies are being used to decrease the malaria burden. Most prominent among them are insecticide treated bed nets (ITN), indoors residual spraying (IRS), artemisinin combination treatments (ACT)(Ogbonna and Uneke ; Lengeler 2004; Beier, Keating et al. 2008) and intermittent preventive treatment strategies in pregnant women or infants(Meremikwu, Donegan et al. 2008). Adequately and simply measuring malaria endemicity is thus of prime importance in order to plan and monitor effectiveness of these control interventions.

Different tools and metrics have been used for more than 150 years to investigate the level of malaria endemicity in a given population. Traditionally, the spleen rate (SR), i.e. the prevalence of splenomegaly in a population, has been used to classify malarial endemicity(Dempster 1848 (reprint in 1930)). This measure however suffers from a lack of specificity. Indeed, many diseases highly prevalent in the tropics may also lead to splenomegaly like visceral leishmaniasis or schistosomiasis. More importantly, it has been shown in Papua New Guinea (PNG) by Brabin *et al*. that the spleen rate may vary between sub-populations of non-pregnant women with similar levels of exposure to malaria(Brabin, Brabin et al. 1988), probably due to differences in immune response to malaria. Splenomegaly can also be difficult to measure reproducibly.

In the 1950's, the prevalence of blood stage malaria parasites (parasite rate for all species, PR) was introduced as the principal metric for monitoring malarial endemicity and remains the method of choice(Metselaar and Van Theil 1959). This metric is much more specific but tends to be influenced by seasonal

fluctuations and is strongly age-dependent. In addition, it requires blood collection and expert microscopy that may not be available in resource-limited settings. Utilizing both SR and PR in children 2-10 years, a classification of malaria endemicity was proposed by WHO and revised by Metselaar *et al*(Metselaar and Van Theil 1959): hypoendemic if SR 2-10 or PR 2-10 is <10%, mesoendemic if SR 2-10 or PR 2-10 is 10-50%, hyperendemic if SR 2-10 or PR 2-10 is 51-75% and holoendemic if SR 2-10 or PR in children < 1year is >75%. These measures may provide good estimates of malaria endemicity in areas where malaria is highly prevalent, but they are less accurate when malaria transmission is lower(Yekutiel 1960) such as in an aggressive control program. The Annual Parasite Incidence (API = number of malaria cases/population, usually expressed per 1000, a malaria episode is confirmed by a positive blood slide) is thus often used in areas of lower transmission. However, this metric requires even more resources than measuring PR including a reliable reporting system for malarial episodes.

All these different metrics have been integrated into control strategies such as the Global Malaria Eradication Programme coordinated by the World Health Organization (WHO) since the mid 1950's. More recently the Malaria Atlas Project (www.map.ox.ac.uk), used the *Plasmodium falciparum* (*Pf*) parasite Rate (*Pf*PR), usually in children 2-10 years(Smith, Guerra et al. 2007), and *Pf* Annual Parasite Incidence (*Pf*API) to map *Pf* endemicity. As part of this work, Hay and colleagues published an extensive and detailed review on the tools and strategies available to monitor malaria endemicity (Hay, Smith et al. 2008).

Given the limitations of the traditionally used measures of malaria endemicity outlined above, there is therefore a need to identify additional easily implementable tools for the accurate measurement of malaria endemicity in a variety of transmission settings.

It is well known that one of the main clinical features of malaria at an individual level is a fall in hemoglobin (Hb), often resulting in anemia (Hedberg, Shaffer et al. 1993; Slutsker, Taylor et al. 1994 ; Price, Simpson et al. 2001). However, little is known of how Hb varies at a population level relative to changes in malaria transmission intensity. Two studies have shown associations between altitude variations and changes in malaria prevalence, with *Pf* prevalence decreasing with altitude(Akhwale, Lum et al. 2004; Drakeley, Carneiro et al. 2005). In the same studies, Hb concentrations of individuals were also found to increase with altitude. This suggests that both the population hemoglobin mean (popHb) and anemia prevalence (AP), following correction for altitude, correlate directly with the variations of malaria transmission intensity and may thus be useful as measures of malaria endemicity. However, the evidence for this direct correlation is lacking as the existing data are limited by the sample size or the number of locations. Furthermore, all studies have been performed in Africa where *Pf* is the predominant species. Therefore, there is a need to investigate the association between Hb measurements at a population level and malaria in larger datasets from areas with a wider range of altitude, malaria prevalence and plasmodium species.

PNG is situated in the South-West Pacific and presents a wide range of malaria endemicity due to its mountainous geography, ranging from highly prevalent at sea level to absent in the highlands (up to 4000 meters)(Mueller, Bockarie et al. 2003). Another characteristic of PNG is that all four species of plasmodium are present, with *Plasmodium falciparum* (*Pf*) responsible for more than 50% of all clinical malaria episodes, and *Plasmodium vivax (Pv)* being responsible for most of the remaining cases. In past surveys, entomological inoculation rate (EIR) has varied between <1 and 400(Mueller, Bockarie et al. 2003). Overall, malaria is the main cause of hospital admissions in lowland areas, but is absent in much of the highlands. Thus, PNG is an ideal site to investigate the relationship and correlation between classical measures of malaria transmission intensity, altitude and variations of population Hb levels and anemia prevalence.

3.3 Methods

Study site and patients

Individual informed consent was obtained from all participants or in case of children their guardians before enrolment into the study. Malaria surveys conducted in the Highlands of PNG (Mueller, Bjorge et al. 2003; Mueller, Taime et al. 2003; Mueller, Kundi et al. 2004; Mueller, Ousari et al. 2006) were performed on behalf of the PNG National Department of Health and the consent was verbal. In the lowlands surveys that were parts of research studies, written consent was obtained. All participants specifically consented for a finger prick blood sample to be collected and assessed for the presence of malaria and anaemia. This Consent process was approved by the PNG Medical Research Advisory Council of Papua New Guinea (respective approval numbers are: Highlands: 00.26, Sepik: 05.08 and Madang: 05.20)

The study was performed in PNG alongside a series of household malaria surveys performed in three different regions of the country: the Highlands (inland, 500 – 2100m), the Middle Sepik (lowland, <500 m) and Madang (coastal, <500m). The different villages were chosen according to their location and representativeness in terms of presumptive malaria endemicity and population diversity. Further details of the selection of Highlands and Sepik survey locations and of the survey methodology are given by Mueller *et al*. (2003-2009) (Mueller, Bjorge et al. 2003; Mueller, Taime et al. 2003; Mueller, Kundi et al. 2004; Mueller, Ousari et al. 2006; Mueller, Sie et al. 2007; Mueller, Yala et al. 2007; Mueller, Widmer et al. 2009; Maraga, Plüss et al. in press). In short, within each village a household based survey was conducted using convenience sampling. All members of a participating household were enrolled in the surveys after individual consent. Medical history of malaria (history of fever, treatment for malaria and other spontaneously reported symptoms) and a brief medical examination including spleen measurement (using Hackett's classification), axillary temperature and targeted clinical examination were performed. All patients presenting with signs or symptoms of malaria had a rapid diagnostic test performed, and malaria treatment was provided if positive. A finger prick was

performed on each participant, blood slides with thick and thin films for malaria microscopy were made and Hb was measured using a portable Hemocue 201® machine (Angelholm, Sweden) in g/dl. The Madang surveys were conducted in the context of preparatory works for a trial of intermittent preventive treatment in infants (IPTi), using the same methodology. Surveys conducted in villages with epidemic outbreaks of malaria were not used for this study.

All blood smears were stained with 2.5% buffered Giemsa (pH 7.2) for 35 minutes and examined by light microscopy. Slides were declared negative if no parasites were seen in 100 thick film fields by two different microscopists. The parasite species in positive films were identified and densities were recorded as the number of parasites/200 WBC. Densities were calculated assuming 8,000 WBC/µl (Genton, Al Yaman et al. 1995). All slides with densities less than 200/µl, along with a randomly selected 20% of all blood films were routinely re-examined. If less than 80% concordance was achieved between evaluations, the entire batch of slides was re-read.

There is no standardized cut-off value for defining anemia for individuals (Beutler and Waalen 2006) in tropical countries. In order to calculate the prevalence of anemia (AP) for each community, we defined a cut-off value of 11g/dl which is clinically meaningful, easy to interpret from a public health perspective and gives a good proxy to anemia (see supplementary material). Crude and adjusted population hemoglobin mean values (popHb) were calculated for all communities. Adjustment was done for sex, age and altitude. Sex and age were adjusted using non-parametric regression splines. Hb was adjusted for altitude using the model published by the CDC (CDC 1989; CDC 1998; Ruiz-Arguelles 2006) with the following formula calculating the correcting factor for the different Hb values: $\Delta Hb\ [g/L] = -0.32 \times (altitude[m] \times 0.0033) + 0.22 \times (altitude[m] \times 0.0033)^2$.

All metrics (popHb, AP, SR and PR) were calculated for both the general population and children 2-10 years. Indeed, spleen rate and parasite rate are usually calculated in this age group for defining malaria endemicity categories(Metselaar and Van Theil 1959).

Statistical methods

All data were analyzed using STATA software, version 10.0. Correlations between the different metrics and altitude were investigated by calculating Pearson's correlation coefficients (r^2); significance was assessed by calculating p-values. The same software was used to draw a graph of the correlations between the different metrics weighted for the number of individuals per village. We performed also an ANOVA analysis to investigate the variation of the different metrics across altitude strata. As both Hb metrics and prevalence of infection are subject to measurement and sampling errors, major axis regression (Smith 2009) was used to determine the symmetric relationship between Hb metrics and prevalence of infection in children 2-10 yrs. The resulting relationships are bi-directional, i.e. prevalence of infection can be predicted from Hb metrics and vice versa All analyses were done on data aggregated at the village/population level and did not include measures of individual level variation.

3.4 Results

21,664 individuals from 156 different communities were surveyed from the Highlands, Madang and Sepik areas, altitudes ranged from 5 to 2120m above sea level. On average there were 143 participants in a village (range [35 - 387]). Overall, the median for age was 14.9 years (range [0 - 99]) with 51.4% of participants female. Among the villages, 27 were at elevations below 500m, 17 were between 500 and 999m, 55 were between 1000 and 1499m and 57 were above 1500m altitude. The ratio of Pf/Pv prevalence rates among the 145 villages is 2.04 (range [0 - 17])

The PR in 2-10 ($F_{3,\ 148}$= 75.6, $p < 0.001$) and SR 2-10 ($F_{3,147}$= 47.0, $p < 0.001$) were found to decrease significantly from 58.1 % and 37.8% in villages < 500 m to 4.3% and 1.6% in those >1500 m. AP also decreased significantly with altitude (<500 m: 62.8%, >1500 m: 12.3%, $F_{3,143}$= 114.0, $p < 0.001$) while PopHb

increased (altitude adjusted: <500 m 10.5g/dl, >1500 m 12.8 g/dl, $F_{3,148}$= 74.3, p < 0.001). Complete altitude stratification data are shown in **Table 3.1**.

Overall, very high correlations were observed between altitude and the different metrics for malaria endemicity, as shown in table 2, both in the general population: PR = -0.80, SR = -0.60, crude popHb =0.83 and AP = -0.85 and in children 2-10 years: PR 2-10 = -0.77, SR 2-10 = -0.68, crude popHb 2-10 = 0.73 and AP 2-10 = -0.81 (all correlations: n = 156, p < 0.001). Similarly high correlations were found between PR (overall and in children 2-10) and other metrics (**Table 3.2**, p < 0.001). Plots of individual measures however do reveal differences between the different measures (**Figure 3.1**).

PopHb and AR decrease linearly at altitudes above 500 m but do vary less at lower altitudes, while at altitude > 1500 m rates of enlarged spleens were very low. Accordingly, the strength of the association between the different metrics varied across altitudinal strata (**Table 3.3**). The correlations between Hb metrics (popHb and AP) and PR 2-10 were strongest between 1000-1499 m altitude, and disappeared above 1500 m: r^2 < 500 m: -0.42 & 0.48, 500 – 999 m: -0.53 & 0.43, 1000 – 1499 m: -0.62 & 0.68 and >=1500 m: -0.14 & 0.2.

Using major axis regression, the following relationships were found to best describe the (linear) relationship between Hb metrics and the prevalence of infections in children 2 to 10 years and Hb metrics: P_{2to10} = 0.854 * AP – 0.162 and P_{2to10} = -0.204 * PopHB + 2.73.

<u>Figure 3-1:</u> Scatter plots of correlations with altitude and Parasite Rate 2-10 years for different metrics.

The different metrics are: Population hemoglobin mean (PopHb), Anemia Prevalence (AP), both in general population and 2-10 years and Spleen Rate in 2-10 years (SR 2-10). Each circle represents one village.

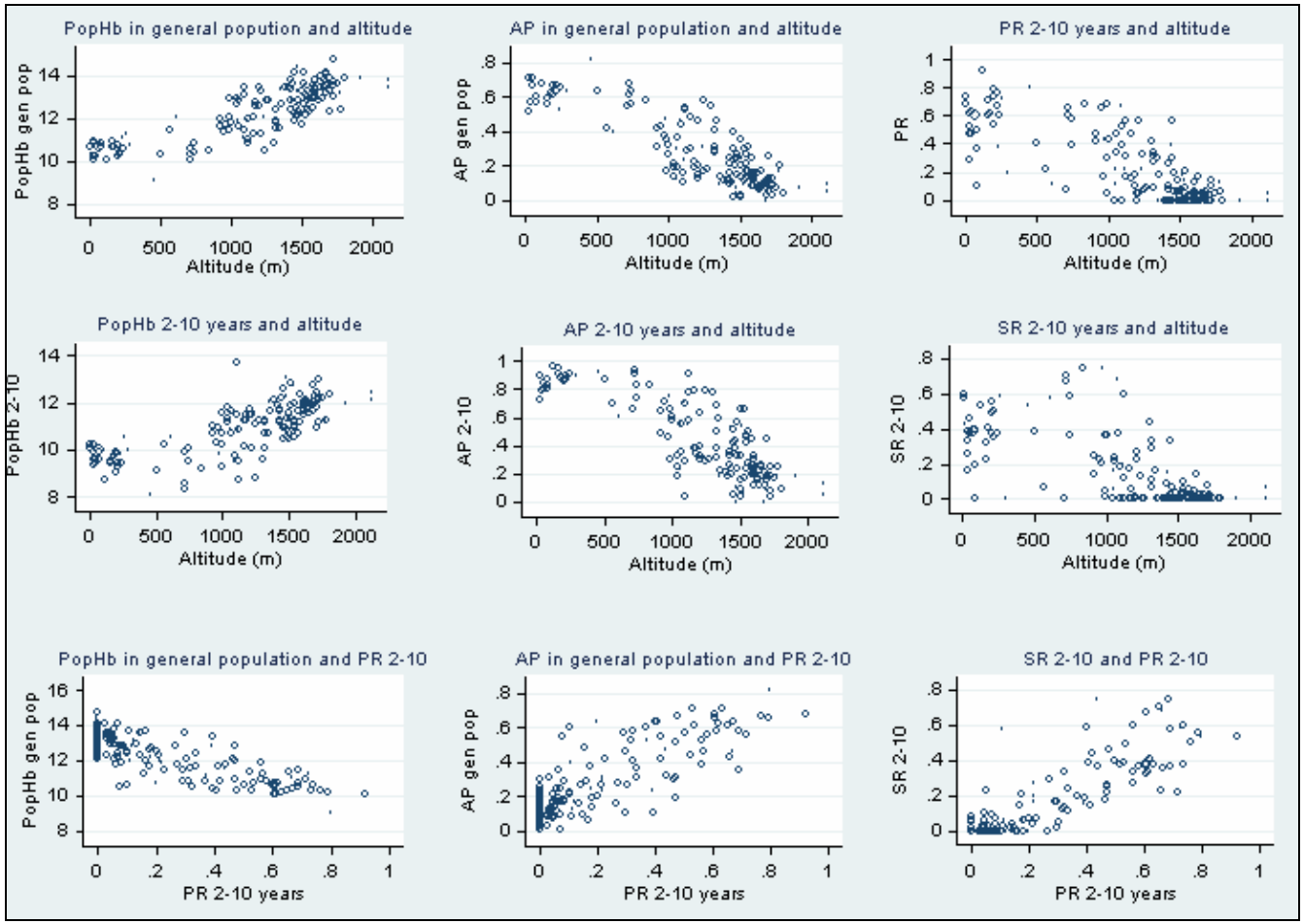

Table 3-1: Metrics for village malaria endemicity by altitude. AP = anemia prevalence, SR 2-10 = spleen rate in children 2 to 10 years and PR 2-10 = parasite rate in children 2 to 10 years.

Altitude	< 500m			500 - 999 m			1000 - 1499 m			> 1500m			
		n=27			n = 17			n=55			n=57		p
	Mean	CI95%	range	**Mean**	CI95%	range	**Mean**	CI95%	range	**Mean**	CI95%	range	
PopHb (crude)	**10.5**	10.4-10.7	9.0-11.3	**11.2**	10.9-11.6	10.0-12.9	**12.4**	12.2-12.6	10.5-14.4	**13.3**	13.1-13.4	11.7-14.7	< 0.001
PopHb Adj*	**10.5**	10.4-10.7	9.0-11.3	**11.2**	10.8-11.5	10.0-12.8	**12.2**	11.9-12.4	10.2-14.1	**12.8**	10.4-10.7	11.4-14.2	< 0.001
AP	**0.63**	0.60-0.66	0.52-0.82	**0.46**	0.39-0.53	0.17-0.68	**0.25**	0.21-0.29	0.01-0.58	**0.12**	0.1-0.14	0-0.35	< 0.001
SR 2 - 10	**0.38**	0.31-0.44	0-0.6	**0.36**	0.23-0.49	0-0.75	**0.12**	0.08-0.16	0-0.68	**0.02**	0.01-0.02	0-0.14	< 0.001
PR 2- 10	**0.58**	0.51-0.65	0.1-0.92	**0.41**	0.30-0.51	0.05-0.69	**0.2**	0.15-0.25	0.0-0.68	**0.04**	0.03-0.06	0.0-0.21	< 0.001

* adjusted for altitude

Table 3-2: Summary of Pearson's coefficients of correlation (r^2) between altitude (Alti), population haemoglobin mean (PopHb) crude or adjusted (for age, sex and altitude), anaemia prevalence (AP), parasite rate (PR) and spleen rate (SR) in the general population (Pop) and children 2-10 years, in non-epidemic context*. AP = anemia prevalence, SR = spleen rate and PR = parasite rate.

		Crude PopHb (pop)	Crude PopHb (2 10 y)	Adjusted PopHb	AP (pop)	AP (2-10 y)	PR (pop)	PR (2-10 y)	SR (pop)	SR (2-10 y)
Alti		0.83	0.73	0.78	-0.85	-0.81	-0.8	-0.77	-0.6	-0.68
Crude PopHb	Pop		0.94	0.99	-0.98	-0.95	-0.79	-0.81	-0.67	-0.75
	2-10 years			0.95	-0.93	-0.95	-0.7	-0.77	-0.65	-0.75
Adjusted PopHb	Pop				-0.97	-0.94	-0.77	-0.79	-0.65	-0.74
AP	Pop					0.96	0.79	0.81	0.67	0.76
	2-10 years						0.75	0.79	0.65	0.74
PR	Pop							0.96	0.71	0.82
	2-10 years								0.76	0.86
SR	Pop									0.93
	2-10 years									

* All coeficients have a p<0.001

Table 3-3: Correlations between different measures of malaria endemicity by altitudinal strata. AP = anemia prevalence and PR 2-10 = parasite rate in children 2 to 10 years.

Altitude	< 500m			500-999m			1000-1499m			>= 1500m		
		n=154			n=147			n=151			n=152	
	HbPop	AR	SR 2-10	HbPop	AR	SR 2-10	HbPop	AR	SR 2-10	HbPop	AR	SR 2-10
PR 2-10	-0.42**	0.48**	0.83*	-0.53**	0.43***	0.55**	-0.62*	0.68*	0.32*	-0.14***	0.2***	0.32**
HbPop		-0.93*	-0.29***		-0.96*	-0.52**		-0.96*	-0.6*		-0.9*	-0.19***
AR			0.52**			0.62*			0.65*			0.18***

** p<0.05, ***p>0.05

3.5 Discussion

Using a very large data set from Papua New Guinea, this study has investigated the correlation between altitude, commonly used metrics (spleen rate and parasite rate in children 2 -10 years and records of febrile illnesses) and hemoglobin levels expressed as population mean hemoglobin (PopHb) and prevalence of anemia (AP) per community. As shown in earlier studies in Tanzania and Kenya (Akhwale, Lum et al. 2004; Drakeley, Carneiro et al. 2005), in PNG PR and SR were strongly correlated with altitude indicating that altitude is a reasonable proxy for malaria endemicity in PNG. In the present study, it was observed that both PopHb and AP were also strongly associated with altitude variations. Crude measures of Hb performed as well as those that were adjusted for effects of age, gender and altitude. Hb measurements were also closely correlated with PR and SR.

In an altitude-stratified analysis, a progressive increase of PopHb is observed, from 10.5 g/dl on average below 500 m, to 12.8 g/dl above 1500 m, after correction for altitude. Of factors other than altitude itself which may explain these variations, malaria is likely to be the most important one. Other “prevalence” metrics (AP, SR 2-10 and PR 2-10) all show progressive decreases with increasing altitude. Correlation of Hb metrics and PR 2-10 are stronger at altitude between 500 and 1500 m, where endemicity is higher (see **Figure 3.1** & **Table 3.3**).

By nature, single cross-sectional surveys are not well suited to describe malaria endemicity in areas of significant seasonality. Although Hb level also show some seasonal variations (Koram, Owusu-Agyei et al. 2003), they are more strongly associated with the history of parasitaemia preceding the Hb measurement than with concurrent parasite densities (McElroy, ter Kuile et al. 2000). Mean Hb levels thus are a measure of mean exposure in the preceding 3 months rather than representing a single time point like parasite prevalence rate. Nevertheless,

when comparing Hb metrics between areas or over the time of an antimalarial intervention, it is important to either conduct surveys during the same period of the year or to adjust for seasonal variations in the relationship between malaria and Hb levels.

Throughout PNG *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* are co-endemic. In most areas *P. falciparum* is the most common infection followed by *P. vivax* and in particular in highly endemic areas mixed species infection are very common (Kasehagen, Mueller et al. 2006; Mueller, Widmer et al. 2009). While infections with any species can result in anemia, *P. falciparum* infection tend to be associated with a stronger reduction in Hb levels (Mueller, Sie et al. 2007; Mueller, Yala et al. 2007; Maraga, Plüss et al. in press) and the higher risk of severe anemia (Wildig, Michon et al. 2006; Genton, D'Acremont et al. 2008) than *P. vivax*. However, as prevalence of infection with all species increases in parallel with increasing endemicity, it is difficult to attribute parts of the observed effect of malaria on Hb levels to individual species. Consequently, overall prevalence rates show stronger association with Hb metrics than either *P. falciparum* or *P. vivax* specific prevalence rates (data not shown). Finally, it is likely that increasing drug resistance to malaria infections will not have the same effect on the different metrics. A recent study in the Gambia showed higher rates of severe anemia in children infected with chloroquine resistant *P. falciparum* (Meerman, Ord et al. 2005). Similarly, the high rates of severe anemia observed in both hospitalized *P. falciparum* and *P. vivax* patients in Indonesian Papua was linked to the very high rate of drug resistance (Tjitra, Anstey et al. 2008). It is thus possible that that an increase in drug resistance might change the pattern of clinical malaria episodes and that consequently Hb levels might be affected differently from PR.

Besides malarial infections there are a number of different factors that also impact on Hb levels. Of these, it is straightforward to control for altitude, sex and

age. As shown in this study, such adjustment may however not necessarily improve the strength of the association between Hb and malarial endemicity.

Other important contributors to the burden of anemia include infections, nutrition status and host genetic traits such as common red blood cell polymorphisms. Among infections, hookworm infestation is probably the most important in PNG. It is highly prevalent throughout the country (Pritchard, Quinnell et al. 1990; Barnish 1992). Although high egg loads may be associated with anemia in some populations of PNG(Shield, Scrimgeour et al. 1980), other studies did not find such a link (Pritchard, Quinnell et al. 1990). Adjusting for the presence of helminth infections would thus potentially improve the accuracy of Hb metric. Unfortunately these data are not available for the PNG surveys. Given the nation-wide presence of hookworm and the lack of consensus on its impact on Hb levels in PNG it is however unlikely that this would have substantially changes the observed relationships between Hb and malaria at the population level. Parvovirus B19 infection has recently been identified as a significant risk factor for severe anemia in PNG(Wildig, Michon et al. 2006), but as an infection of young children with limited variation between populations(Wildig, Mueller et al. 2007) the effect of B19 infection will mainly affect this age group and to a lesser extent the adult population. To-date, no studies have investigated the overall contribution of B19 infections to the burden of anemia. Schistosomiasis, which contributes a lot to the burden of anemia elsewhere, is not present in PNG. In other malaria endemic areas like Africa, it will however be necessary to take into account other infectious agent. In particular, Schistosomiasis as an important confounder and adjustment might thus be required where it is endemic. In conclusion, whereas co-infections might potentially affect population Hb levels, the actual impact will depend on epidemiological context and due to the lack of appropriate co-infection data is often difficult to quantify.

Secondly, dietary factors such as malnutrition or micronutrient deficiencies impact on Hb levels. A recent review by Metz has shown that folate and vitamin

B12 deficiency, even though very frequent in developing countries, do not significantly affect the prevalence of anemia(Metz 2008). Vitamin A (by affecting erythropoiesis(Cusick, Tielsch et al. 2005)) and Iron deficiencies are the two micronutrients which are contributing the most to anemia. Low levels of Vitamin A may also contribute to an increase risk of malaria (Shankar, Genton et al. 1999). In the 2005 PNG National Malnutrition survey 15% of children <5 years had low levels of VitA with little regional difference. VitA deficiency was however almost completely absent in adults (Saweri, pers. communication). In the same survey 23% of people had elevated transferrin receptor (TfR) levels that were indicative of iron deficiency. Although the prevalence of elevated TfR varied significant with age and among regions, the prevalence of elevated TfR among anemic participants (defined as <10 g/dl for children < 11 for adults) was similar in children and adults (42-47%), albeit lower in highlands (19-32%) compared to lowlands population (38-64%). While chronic protein energy malnutrition and resulting growth stunting is highly prevalent throughout PNG (Mueller, Vounatsou et al. 2001), acute malnutrition is less common overall but more important in the coastal areas than in the highlands (Edwards 2000). The relationship between protein-energy malnutrition, malaria and anemia is complex and not fully understood. Some recently published data suggest that malnutrition contributes to both malaria associated morbidity and anemia (Caulfield, Richard et al. 2004; Friedman, Kwena et al. 2005; Ehrhardt, Burchard et al. 2006) while an earlier study in lowlands PNG found a reduced risk of malaria in stunted children (Genton, Al Yaman et al. 1998). However, in studies in highly endemic areas where both have been assessed malaria is usually a much stronger contributor to anemia than malnutrition(Ronald, Kenny et al. 2006). Whereas adjusting for nutritional status might thus improve the accuracy and predictive value of Hb metrics, such data is rarely collected in malariological surveys.

Finally, there are a number of genetic traits that are known to impact on hemoglobin levels. By far the most important one in PNG is alpha-thalassemia 2 ($-\alpha$) which is very common in lowlands PNG but virtually absent in highlands

populations(Serjeantson 1992). This form of alpha-thalassemia only causes mild symptoms and even in its homozygote presentation, it has only a minor effect on hemoglobin levels(Weatherall and Clegg 2001). The homozygote however has a significant protection against severe malaria (including severe malarial anaemia) and severe non-malarial illnesses(Allen, O'Donnell et al. 1997). Thus, a potential negative effect of alpha-thalassaemia on population Hb may be off-set by a reduction in risk of malaria associated (severe) anemia. Its overall impact on popHb and AR is likely to be limited. The other two common red blood cell polymorphisms are South East Asia Ovalocytosis(Genton, Ai-Yaman et al. 1995) and the Gerbich blood group(Sheral, Christopher et al. 2004). Although both traits are restricted to malaria endemic areas(Mgone, Koki et al. 1996), they both cause little or no difference in Hb levels(Palek and Jarolim 1993). Sickle cell anemia is not present in PNG. Consequently, the impact of host genetic polymorphisms on the association between Hb levels or anemia prevalence and malaria exposure is likely to be minor in PNG. Importantly, changes in malaria transmission intensity will occur much more rapidly than those in frequency of host genetic polymorphisms. Thus, even if such traits may affect Hb levels, changes in popHb or AR over time would still correctly reflect changes in malaria endemicity.

After consideration of these important potential confounders, the correlations between Hb metrics and altitude, parasite prevalence and spleen rates appear to remain robust. This study therefore provides good evidence that popHb and AP are valuable metrics to estimate the burden of malaria in PNG even without adjusting for potential confounders. Before applying them to other settings, in particular those where schistosomiasis is highly prevalent, it will be important to cautiously consider the impact of such confounders when interpreting Hb levels as malaria metics.

The major advantages of using Hb metrics as measures for malaria endemicity is that they require limited resources to be recorded, they are immediately available

in the field and are more reproducible than estimating spleen rate. In addition, popHb is very easy to calculate and AP easy to interpret. Last but not least they also provide important additional information on the health status of a population.

Hb metrics thus appear to be promising alternative tools for measuring malaria burden, on their own or in combination with other traditional or more recent serological(Shekalaghe, Alifrangis et al. 2009) measures of malaria endemicity, even in areas of high prevalence of non-falciparum infections. They may be particularly useful in areas of moderate to low transmission and could therefore be of best use in public health when monitoring malaria control interventions programs during the preparatory and attack phases(Hay, Smith et al. 2008). Additional in-depth studies are warranted to better characterize the relationship between Hb levels and malaria transmission and determine the optimal use as new malaria metrics. In addition, prospective studies in areas with different malaria epidemiology and in the context of malarial control initiatives are required. The examination of changes in Hb-based indices over time in populations benefitting from a major anti-malaria intervention would comprise an important test of the usefulness of Hb metrics based endemicity monitoring in the context of rapidly changing malarial epidemiology in Papua New Guinea or elsewhere.

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4 Unified treatment with artemether-lumefantrine based on RDT results: an effectiveness study in PNG infants with *P. falciparum* and *vivax* malaria

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4.1 Abstract

Objectives

The management of febrile children in malaria endemic areas has evolved and the following approach has been proposed: febrile children should be tested for malaria [rapid diagnostic test (RDT) or blood slide (BS)], and receive effective malaria treatment only if positive. There are safety concerns about withholding antimalarial drugs from children with negative tests. More generally, there is limited evidence on the safety and feasibility of this strategy, as well as no evidence of ACT effectiveness as unified treatment in areas with mixed endemicity [*Plasmodium falciparum* (*Pf*) and *P. vivax* (*Pv*)]. The present study explores the feasibility of this approach in Papua New Guinea (PNG), a highly endemic country for both *Pf* and *Pv*.

Design

longitudinal prospective study

Setting

Outpatient clinics, Madang and Sepik provinces, Papua New Guinea

Participants

1605 infants 3 months old enrolled and followed prospectively for up to 2 years

Main Outcome Measures

Rates of re-attendance and occurrence of serious adverse events/complications

Results

Among 7211 febrile episodes, 5670 fulfilled the inclusion criteria (fever and/or history of fever in the past 48h, no malaria treatment within 4 weeks, no previous fever within 2 weeks, no critical illness and no malaria treatment if RDT neg at D0). The mean duration of symptoms was 2.7 days (95%CI 2.6-2.8). 3942 (70%) had a negative RDT. Among them, 133 (3.4%) re-attended the clinic within 7 days for fever of which 29 (0.7%) had an associated parasitaemia detected by RDT or microscopy. Of the 133, 1 died of lower respiratory tract infection (LRTI) with negative RDT & BS. 23 (0.6%) infants re-presented with a serious adverse event: two had LRTI with low densities *Pv* on BS, 2 were diagnosed as *Pv*

malaria based on RDT but BS were negative, 15 had LRTI and 3 had alternative diagnoses. Of these, 22 were cured and 1 died while admitted for possible meningitis (RDT and BS negative).

There were 1728 children with positive RDT results. All infants were treated with artemether-lumefantrine. 30 (1.7%) re-attended within 7 days for fever, none died and 5 (0.3%) developed a serious adverse event, all with a likely diagnosis of malaria.

Conclusion

This study provides solid evidence that rapid diagnostic testing for malaria and treatment with ACT for positive cases is safe and feasible in infants leaving in areas with high endemicity for both *Pf* and *Pv* infections.

4.2 Introduction

In many countries, patients are treated presumptively for malaria when presenting with febrile illnesses. This is because (1) no diagnostic tools are available (microscopy or rapid diagnostic tests), (2) health workers do not trust the results of these tests(Reyburn, Mbakilwa et al. 2007; Zeno, Bienvenu Sodiomon et al. 2009) or (3) there are scarce data on the safety of withholding antimalarial drugs in patients with a negative test for malaria. This is especially true in children under five, in whom presumptive antimalarial treatment has been promoted by the World Health Organization (WHO) (WHO 2009) for decades.

The situation has evolved and more tools and data are now available for the management of suspected malaria episodes, in particular in Sub-Saharan Africa(D'Acremont, Lengeler et al. 2009). First, community awareness in endemic areas of the importance of early attendance at a health facility when a child is febrile has improved.(Sanjana, Barcus et al. 2006) Second, reliable and affordable Rapid Diagnostic Tests for malaria (RDT) are now widely available.(Bell, Wongsrichanalai et al. 2006; Shillcutt, Morel et al. 2008; WHO 2008; Drakeley and Reyburn 2009) Third, safe and highly efficacious treatments for malaria (artemisinin-based combination therapies, ACT) are available in almost all endemic countries. A review by Douglas et al. (Douglas, NM. et al. 2010) looking at the appropriateness of a single ACT-based strategy for both *Plasmodium falciparum (Pf)* and *Plasmodium vivax (Pv)* in co-endemic regions, a so-called unified treatment, concluded that it might be a reasonable option where *Pv* is resistant to chloroquine. However, these conclusions are based on a small number of highly controlled efficacy trials, and data are lacking on the effectiveness of this strategy in routine settings. Last, one recently published study has demonstrated the safety of withholding antimalarial drugs in children with negative RDT under five in Tanzania, where *Pf* is the predominant species.(d'Acremont, Malila et al. 2010)

The safety and effectiveness of diagnosis-based treatment of malaria has not been investigated in infants. Moreover, it is not clear whether withholding antimalarial treatment in young children with fever and a negative RDT is safe in

different epidemiological contexts, especially in areas with a high prevalence of *Pv* infections; it is well known that many RDT perform poorly for the diagnosis of this species.(Sun Hyung, Myung-Hyun et al. 2008; WHO 2008; Ashley, Touabi et al. 2009; Meena, Joshi et al. 2009) More data are thus needed to ensure that the new WHO policy of only prescribing antimalarials to children with confirmed parasitemia is safe in all settings(WHO 2010). It must be a priority in terms of clinical management of febrile illnesses as it is well recognized that presumptive treatment for malaria may delay the management of other potentially life threatening diseases and promote the development of resistance to antimalarial drugs.(Reyburn, Mbakilwa et al. 2007)

Papua New Guinea, located in the South Pacific, is a tropical country highly endemic for malaria in costal areas, with malaria incidence rates in small children higher than 1 episode/year/child in most regions, *Pv* accounting for at least half of all infections (Mueller, Bockarie et al. 2003) (Senn *et al*, personal communication). As part of a large trial of intermittent preventive treatment in infants (IPTi), a passive case detection surveillance system was implemented and all sick study participants presenting spontaneously at the study clinics with fever or history of fever were screened with RDT for *Pf* and non-*Pf* malaria infections and treated with ACTs only when the test was positive. The standardized approach to case management employed offers an excellent opportunity not only to investigate the safety of withholding antimalarial drugs in febrile patients with a negative RDT result in a situation where *Pv* infections are very common, but also to assess the appropriateness of using a unified treatment for malaria (i.e. using an ACT indiscriminately for both *Pf* and *Pv*).

4.3 Methods

Background & study sites

This work was carried out alongside a large 3-arm drug trial of intermittent preventive treatment for malaria (IPTi) including more than 1500 children 3–27 months old from 2006 to 2010 (www.ClinicalTrials.gov: NCT00285662). Children

were randomly assigned to a treatment course of sulfadoxine/pyrimethamine (single dose, 25/1.25mg/kg) with 3 days of either 10mg/kg of amodiaquine (SP-3AQ) or 4 mg/kg of artesunate (SP-3AT), or to a placebo group. The doses were given every 3 months during the first year of life, along the immunization schedule. This trial took place in two different settings:

Mugil (Madang province): the main study site, at which 1125 study participants were enrolled, is situated 60km north of Madang town. It is a rural area served by one health center and three small aid posts. It is highly endemic for malaria with an incidence rate of clinical malaria (fever + positive blood slide or RDT) of 0.94 episodes / child / year, *Pv* accounting for two thirds of all infections (unpublished results of IPTi trial).

Maprik: a secondary study site where 480 study participants were enrolled. It is also a rural area, located in the East Sepik Province, about 400 km from Madang. It is served by two sub-health centers (Kunjingini and Kaugia) and one aid post. The incidence rate of malaria is low: 0.13 episode / year / child, *Pf* accounting for two thirds of all episodes (unpublished results of IPTi trial). Because of this low malaria incidence, the study was prematurely stopped at this site in May 2008.

Data collection (passive case surveillance)

A passive case detection surveillance system had been established for 20 years in Maprik (Genton, D'Acremont et al. 2008) and 10 years in Mugil (Michon, Cole-Tobian et al. 2007) for studies who took place before the IPTi trial. The same infrastructures were used for IPTi and when their child was sick, all parents of study infants were asked to visit one of the health facilities. All study patients were reviewed by study nurses working in collaboration with the health centers or aid posts. A standard case report form was filled for each illness episode that included all relevant signs and symptoms as well as the clinical management. When presenting with fever or history of fever in the past 48 hours, children were screened with an RDT for *Pf* and non-*Pf* malaria infections (ICT Combo®, South Africa) and only treated with artemether-lumefantrine (AL, Coartem®, Novartis,

Switzerland) if the test was found positive (oral quinine was administered if the last episode was less than 4 weeks prior to attendance). All study nurses were trained to perform and interpret the RDT. Two thick and thin blood slides (BS) for malaria microscopy and a blood sample for PCR were also collected. Additionally, parents had the possibility to attend voluntarily the mobile clinics that were visiting the different villages on a monthly basis to deliver the immunization and IPTi interventions. On the rare occasions when infants visited a health facility after hours (when the study clinics were closed), they were managed according to the national guidelines by the staff on duty. Usually blood samples were not taken and management was done based on clinical criteria. A review was made by study nurses on the following day.

During the 3-monthly regular visits (at time of immunization and IPTi treatment), the health books were reviewed and all medical attendances outside the study clinics were recorded (including date, diagnosis and treatment). If a child died at home, a verbal autopsy was performed whenever possible.

All procedures followed the Good Clinical Practice (GCP) guidelines, and all parents signed an informed consent form when enrolling their child in the IPTi study. The IPTi protocol was also approved by the Medical Research Advisory Committee of Papua New Guinea (MRAC number 05/20).

Study design and procedures

The present work makes use of morbidity data of the IPTi trial cohort to perform safety assessment of children who had an RDT performed as part of a passive case detection visit.

We applied the following inclusion criteria:

- Children 3-27 months enrolled in the IPTi study in PNG
- History of fever in the previous 48 hours and/or temperature > 37.5°
- No positive RDT result in the previous 4 weeks
- No consultation for history of fever/temperature > 37.5° in the past 2 weeks

- No antimalarial drugs prescribed on the day of consultation if RDT negative on the day of consultation
- No severe illness requiring admission or referral (danger signs/symptoms)

One or more illness episodes could occur for each child enrolled in the study.
For all children with above mentioned inclusion criteria, we determined whether they re-attended any of the study clinics in the following 7, 28 and 42 days. Upon re-attendance, we investigated whether or not they still had fever, and examined the clinical and paraclinical outcomes. These were classified as death, Serious Adverse Event (SAE) or non-severe adverse event (AE) ambulatory. The result of the new RDT performed upon re-attendance was cross-checked by a research BS for all AE, SAE and deaths. For the children who had died or had been admitted elsewhere or after hours, we recorded the presumptive diagnosis, management and treatment from the health book or records from the health center.

Serious Adverse Event (SAE)/complication

Any illness episode was defined as an SAE/complication if it resulted in hospital admission or was life-threatening (one or more danger signs/symptoms was present as defined by PNG National Guidelines, or the study clinician considered the event to be life-threatening on the basis of a medical review) or it fulfilled the hematological criteria for an SAE, according to the standardized IPTi consortium guidelines (internal document, www.ipti-malaria.org) for Grading Severity of Adverse Events (examples: disability such as cerebral palsy following a severe malaria infection or hemoglobin below 5 g/dl).

Interpretation of blood slide (BS) and RDT results

Children enrolled in the IPTi who experienced a febrile episode had an RDT performed to decide on treatment and also a BS for research purpose (not use for clinical management). Thick films were examined by light microscopy (LM) for 100 fields (under 100X oil immersion lens) before being declared infection-negative. Parasite species in positive films were identified and densities recorded

as the number of parasites per 200 white blood cells (WBC). Densities were converted to the number of parasites per ml of blood assuming 8,000 WBC per ml (population average WBC count [3]). The BS's were read by two experienced microscopists. In case of discrepancies of readings (absence/presence of parasite, or more than one log difference for parasite densities), a third independent read was performed. In case the readings were still discrepant, results were compared with PCR results and a 4th read was performed for the BS having a different result from the PCR.

It is well known that discrepancies between BS readings and RDT results can be important due to the fact that the two tests detect different components (antigens for RDT and whole parasites for microscopy). Therefore we designed a matrix to interpret the combination of BS and RDT upon re-attendance (see **table 4.1**). According to this matrix, we defined 4 categories of malaria status upon re-attendance: 1) negative = BS & RDT negative, BS or RDT negative and corresponding BS or RDT absent, 2) Definite malaria = BS & RDT positive, 3) Probable malaria = BS positive and RDT negative or absent, 4) Possible malaria = RDT positive and BS negative or absent. Similarly, we defined the presence of specific parasites: definite *Pf* / Pv / *Pm* / *Po,* probable Pf / Pv / *Pm* / *Po*, Possible Pf / *Pv* / *Pm* / *Po*. Mixed infections were allocated to each present species. For example a BS and an RDT indicating mixed infection for *Pf* and *Pv* was classified as Definite *Pf* and definite *Pv*.

Table 4-1: Matrix of interpretation of BS and RDT results upon re-attendance

		RDT rslt				
		Neg	**Pf**	**Pv**	**poss mixed**	**no RDT**
BS results	**Neg**	Def Neg	Poss Pf	Poss Pv	Poss Pf & Poss Pv	Prob Neg
	Pf	Prob Pf	Def Pf	Prob Pf and poss Pv	Def Pf and poss Pv	Prob Pf
	Pv	Prob Pv	Prob Pv and poss Pf	Def Pv	Def Pv & Poss Pf	Prob Pv
	Pm	Prob Pm	Prob Pm and poss Pf	Def Pm	Def Pm & Poss Pf	Prob Pm
	Mix	Prob Pf and Prob Pv	Def Pf & prob Pv	Def Pv and Prob Pf	def Pf and def Pv	Prob Pf and Prob Pv
	No BS	Poss neg	Poss Pf	Poss Pv	Poss Pf and Poss Pv	no results

no results
Neg
Def malaria
Prob malaria
Poss Malaria

Data analysis

All data were doubled entered using FoxPro software as for the IPTi main trial. All data management & analyses were performed using STATA software (10.0). Re-attendance rates within 7, 28 and 42 days for the different outcomes (SAE, death and mild diseases) were obtained for children fulfilling inclusion criteria.

The risk of symptomatic malaria among participating children may have been lessened by the prophylactic effect of the IPTi intervention. To avoid this potential bias, we excluded from the analysis the children who received an IPTi treatment dose prior to the next attendance. We did also compare children in the treatment group versus those in the placebo group for the rates of re-attendance of SAE's.

All rates are displayed with a confidence interval of 95%. Differences between rates were assessed using a chi square test.

4.4 Results

General description of the cohort

Between June 2006 and May 2010, 1605 children 3-27 months were enrolled and followed-up in the IPTi randomized controlled trial (Madang=1125 and Maprik=480). 1512 (94%) children presented at least 1 illness episode (Madang=1053 and Maprik=459). The morbidity surveillance reported a total of 8944 illness episodes (Madang=5978 and Maprik=2966) (see **figure 4.1 & 4.2)**, of which 7223 were febrile (Madang=5249, Maprik=1974). The average number of febrile episodes per child was 4.8. 13 deaths and 371 SAEs were reported during the observation period. The mean duration of symptoms before attendance was 2.7 days (95%CI 2.6-2.8) in febrile children.

We included in our analysis 5670 febrile episodes (Madang= 4103, Maprik = 1567) fulfilling the inclusion criteria. 1899 occurred in children enrolled in SP-3AQ group, 1888 in the SP-3AT group and 1883 in placebo group. 3942 (70%) of the infants had a negative RDT at inclusion. 2512 (61%, 95%CI, 60 - 63) were negative in Madang (high endemicity) and 1430 (91%, 95%CI, 90 - 93) in Maprik (low endemicity). 1728 (30%, 95%CI, 29 - 32) had a positive RDT for any species

of which 1289 were confirmed by BS. **Table 4.2** displays the correlation between positive RDT result and BS results. According to RDT results, 19% (95%CI, 17 - 21) were positive for *Pf*, 37% (95%CI, 35 - 39) were possibly mixed infections (*Pf* and *Pv, Po* or *Pm*) and 44% (95%CI, 42 - 46) were non-*Pf* infections. Median duration of symptoms was similar for children with negative and positive RDT (2 days, IQ ranges of 1-3 and 1-4 respectively).

Table 4-2: Corresponding BS results for all children with positive RDT results (total = 1728)

		RDT results		
		Pf	**non-*Pf***	***Pf* +/- mixed infection**
BS results (research read)	**Negative**	96	111	76
	Pf	37	10	326
	Pv	159	583	116
	Pm	0	8	0
	Mixed infections (*Pf* & *Pv*)	5	3	42
	No BS	29	43	84

About 20% of children did not undergo blood collection while presenting with an SAE. Reasons included admission after hours when no study staff was present, the SAE occurred at home (especially death), or the parents refused the bleeding.

Clinical and parasitological outcomes upon re-attendance within 7 and 28 days following a negative RDT result

Figures 4.1a displays the outcome within 7 days of febrile episodes for children with a negative RDT result. Among the 3942 children with febrile episode and a negative RDT result, 172 (4.4%) re-attended the clinic within 7 days, of whom 133 (3.4%) had a history of fever and/or temperature >37.5°. 29 out of 3942 (0.7%) children with negative RDT at D0 re-attended the clinic with a parasitemia identified by RDT and/or BS. Among the 133 children who re-attended with fever, 1 died probably of severe respiratory infection (as per clinical assessment); malaria was ruled out by both RDT and microscopy at initial and subsequent

attendance. 23 children (0.6%) presented with an SAE, the median duration between initial negative RDT and SAE being 2 days (IQ range 1 - 4).

<u>Figure 4-1:</u> Flow-charts showing the clinical and parasitological outcomes upon re-attendance within 7 days following a negative RDT

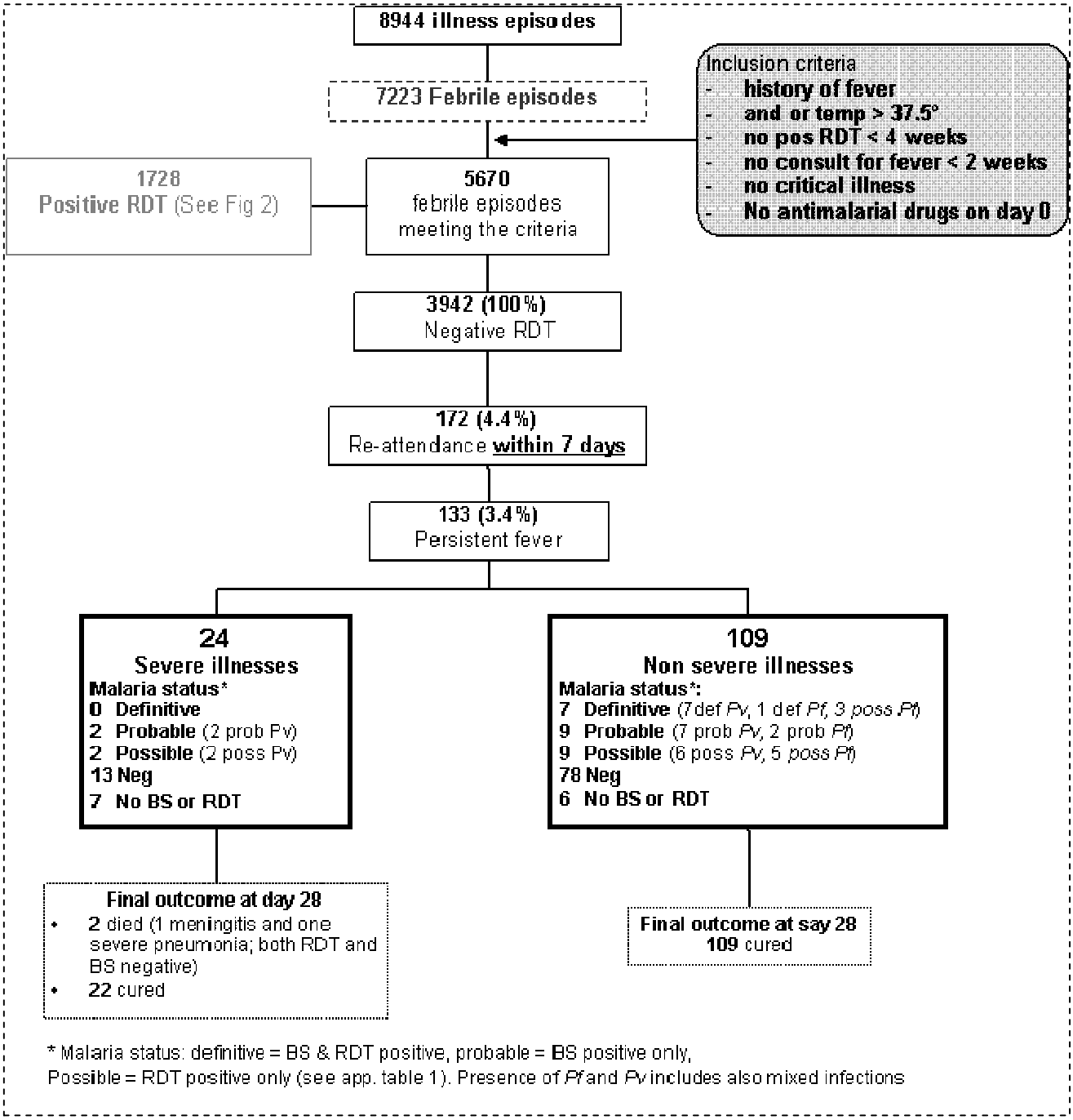


Table 4.3 describes these SAEs in detail. Six children with an SAE had neither an RDT nor a BS performed. This was mainly due to the fact that they were admitted during the night at the health facility when no study staff was present. For these children, the likely diagnosis was made based on clinical records matched with treatment records. Out of the 23 patients re-attending with an SAE, two children were clinically diagnosed as LRTI, and also had low density *Pv* parasitemia (<1000 parasites/µl), but none received antimalarials; and two other children had a positive RDT for *Pv* but the BS were negative. One other child died, one week after admission while being treated for a LRTI, probably of a meningitis (RDT and microscopy were both negative at initial and subsequent visits).

Table 4-3: Details of SAE's at re-attendance within 7 days following a negative RDT for malaria

	RDTm at re-attendance	Blood slide at re-attendance	Antibiotics received	Antimalarial received	Likely diagnosis*	comments
1	Neg	Neg	yes	no	LRTI	Died 7 days later of Meningitis
2	Neg	Neg	yes	no	LRTI	
3	Neg	Neg	yes	no	LRTI	
4	Neg	Neg	yes	no	Malnutrition + LRTI	
5	Neg	Neg	yes	no	LRTI	
6	Neg	Neg	yes	no	Gastroenteritis	
7	Neg	Neg	yes	no	LRTI + Febrile fits	
8	Neg	Neg	yes	no	LRTI	
9	Neg	Neg	yes	no	LRTI	
10	Neg	Neg	yes	no	LRTI	
11	Neg	Neg	yes	no	LRTI	
12	Neg	Neg	yes	no	LRTI	
13	Pos	Neg	no	yes	LRTI	
14	Pos	Neg	yes	yes	LRTI + Gastroenteritis	
15	not done	Neg	yes	yes	LRTI	
16	Neg	Pos (Pv 960/µl)	yes	no	LRTI + Anemia + parasitemia (Pv)	received Co-trimoxazol
17	not done	Pos (Pv 360/µl)	yes	no	LRTI + parasitemia (Pv)	
18	not done	Not done	yes	yes	Skin abscess at injection site	
19	not done	Not done	yes	no	LRTI + Febrile fits	
20	not done	Not done	yes	no	LRTI	
21	not done	Not done	yes	no	LRTI	
22	not done	Not done	yes	no	Tuberculosis	received anti-TB treatment
23	not done	Not done	yes	no	LRTI	

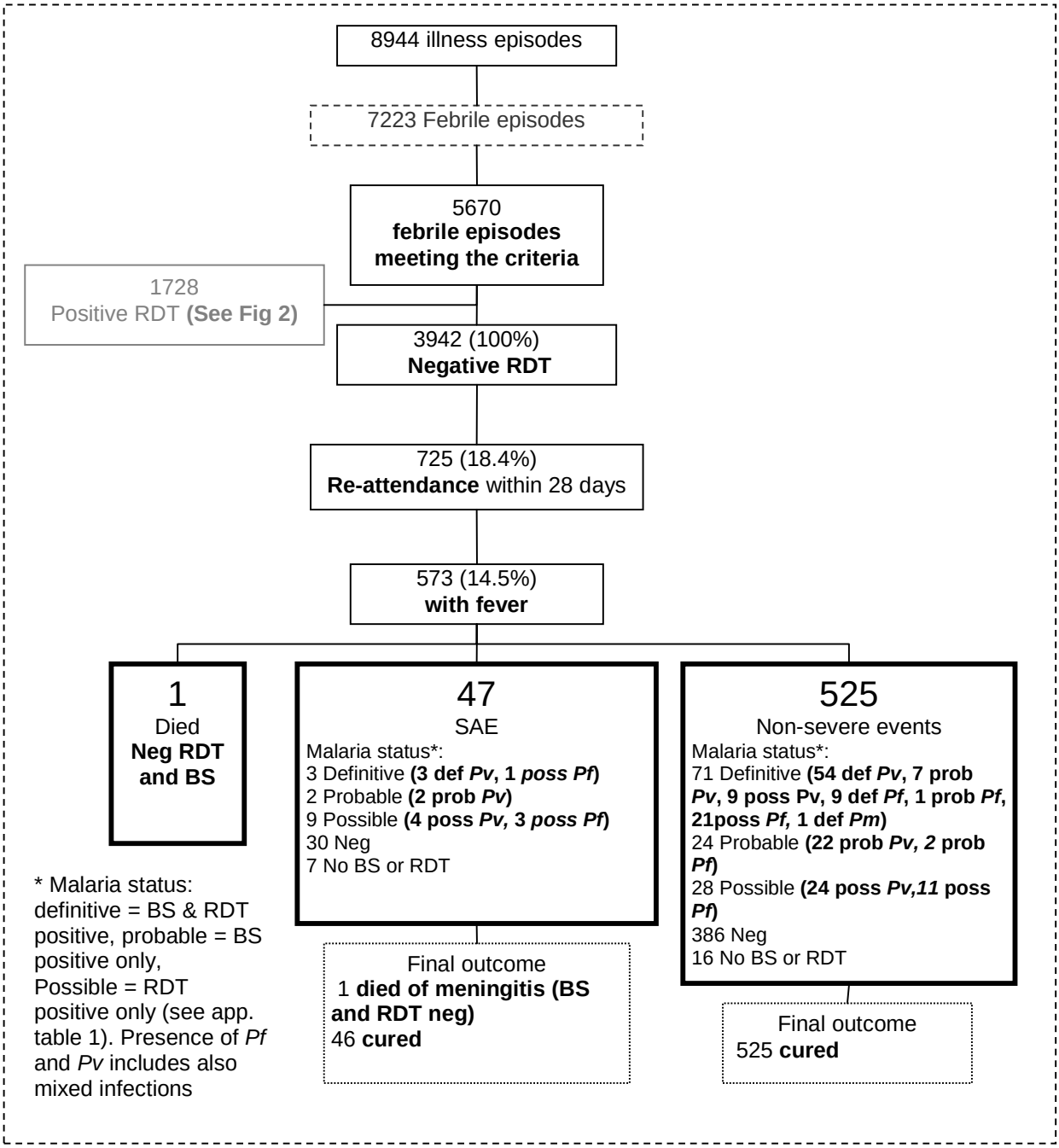
* Likely diagnosis is based on the review of clinical records matched with RDTm & blood slide results and the treatment received

If we looked at the cumulative re-attendance pattern from 0 to 28 days (see **figures 4.2**), 725 infants visited the clinic, 573 had fever, 47 had a SAE and one died. Out of the 47 admissions, the recorded clinical diagnoses were as follow (up to three different diagnoses per child were recorded): 31 LRTI, 10 malaria (according to BS and/or RDT results: 3 definite malaria, 5 possible and 2 negative. Detail species results are displayed in **figure 4.2**), 8 neurological syndromes (febrile fits and/or meningitis), 4 gastro-intestinal diseases, 5 anemia, 2 otitis, 2 URTI, 1 skin infection, 5 other diagnoses.

Figures 4.3 displays the outcome of 1728 out of 5664 febrile episodes(30%) for children who re-attended the clinic within 7 days following a treatment with AL for a positive RDT result. 45 (2.6%) children re-attended the clinic and 30 (1.7%) still had fever. Only 5 infants experienced an SAE in the next 7 days following a positive RDT result, none died. **Table 4.4** shows the details for all SAEs. The median duration between a positive RDT and an SAE was 2 days (IQ range was 1 - 4).

When we extend the observation period following a positive RDT to 28 days (see **figures 4.4**), we observed that 178 infants out of 1728 (10.3%) re-attended the clinic for an ongoing or new fever episode. 162 were non-severe cases. Among the 16 who experienced an SAE (up to 3 different diagnoses were recorded per child), 7 were diagnosed with malaria, 9 with a lower respiratory tract infection, 8 had neurological syndromes (such as febrile fits or meningitis), 3 had anemia and 3 had others diagnoses. One of the children with an SAE died the day after its admission, with the dual diagnosis of LRTI and possible severe malaria. Indeed, this child had a severe respiratory distress syndrome on admission with a possible diagnosis of malaria (positive RDT result but negative BS).

Figure 4-2: Flow-charts showing the clinical and parasitological outcomes upon re-attendance within 28 days following a negative RDT



Clinical and parasitological outcome upon re-attendance within 7 and 28 days following a positive RDT result

Figure 4-3: Re-attendance within 7 days following an initial positive RDT result

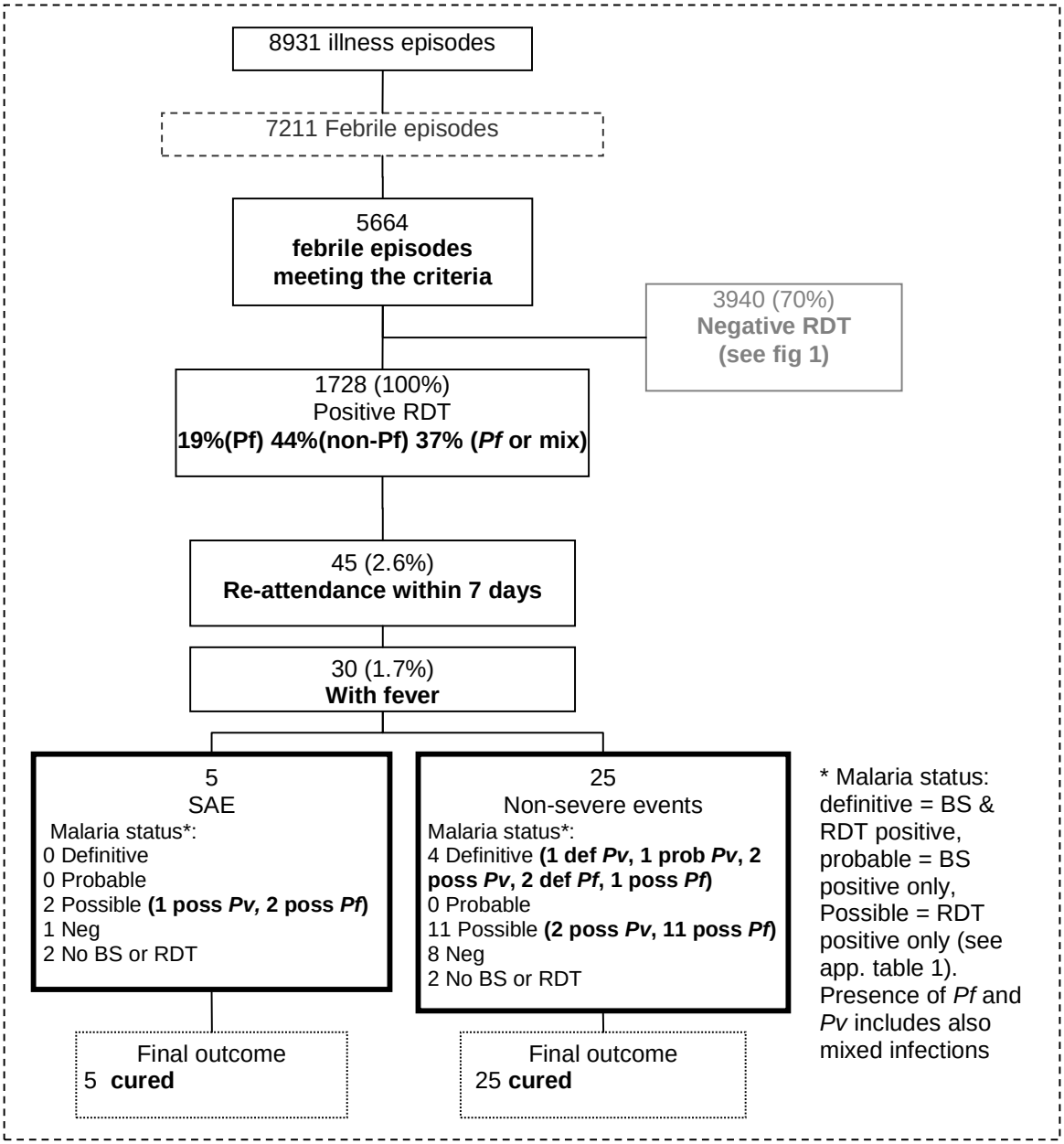


Table 4-4: Details of SAE's at re-attendance within 7 days following a positive RDT for malaria

	RDTm at re-attendance	Blood slide at re-attendance	Antibiotics received	Antimalarial received	Likely diagnosis*	comments
1	Not done	Not Done	Yes	Yes	malaria, febrile fits	
2	Pos (poss mix)	Not Done	No	Yes	malaria + febrile fits	
3	Pos (*Pf*)	Not Done	No	Yes	malaria	Coartem to Quinine
4	Not done	Neg	Yes	Yes	malaria +/- febriel fits	Coartem to Quinine
5	Not done	Not Done	Yes	Yes	Malaria + LRTI	

LRTI = Lower Respiratory Tract Infection

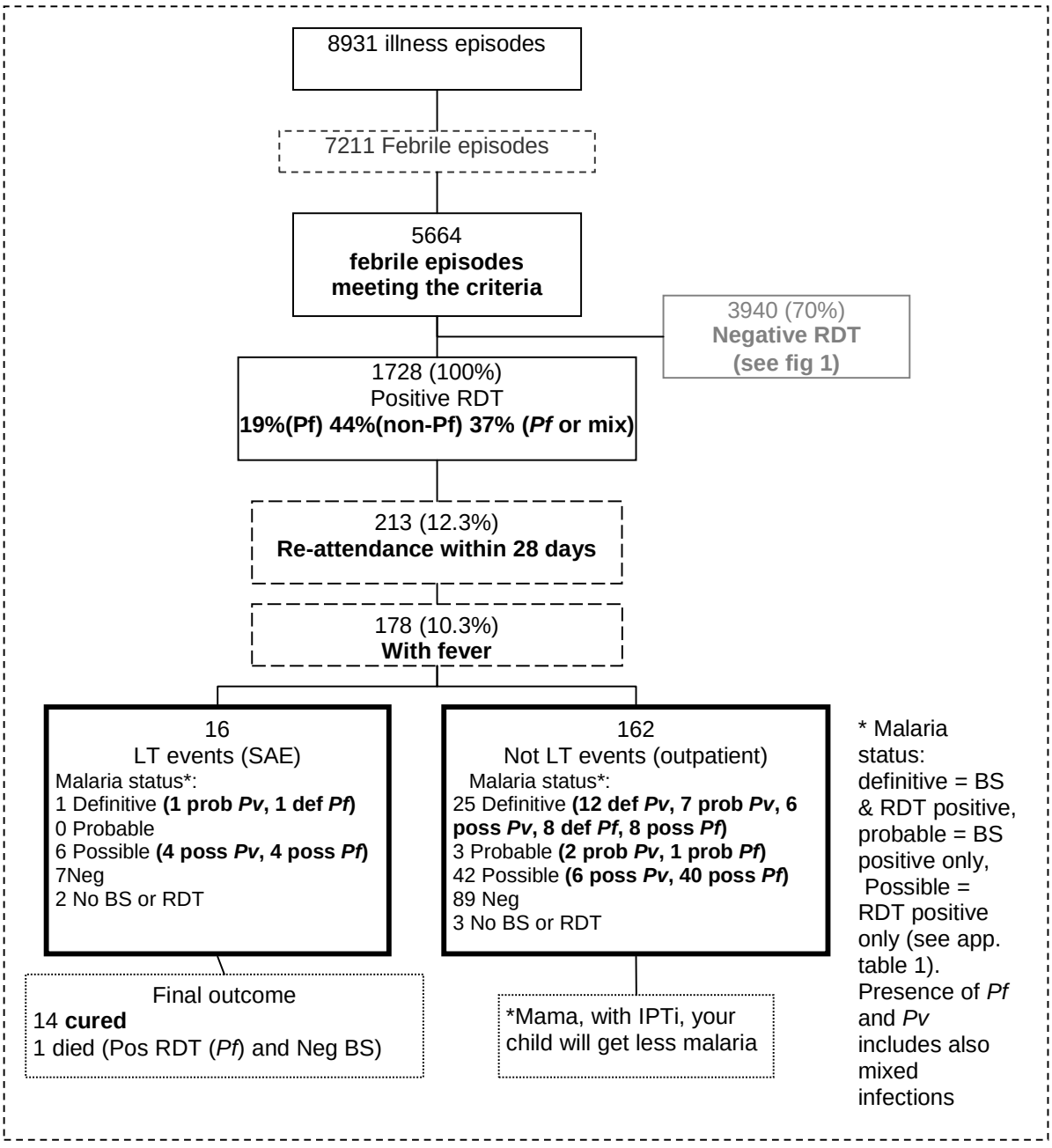
Table 4-5: Crude rates of re-attendance for children having clinical malaria confirmed by RDT and BS and treated with AL presenting with a new clinical malaria due to the same species (PCR uncorrected for re-infections). Def = definitive, Prob = probable, Poss = possible

	Day 0	Re-attendance 0 to 7 days N (%)					Re-attendance 0 to 28 days N (%)					Re-attendance 0 to 42 days N (%)				
	malaria episodes	Def	Prob	Poss	Neg or other species	No BS and RDT	Def	Prob	Poss	Neg or other species	No BS and RDT	Def	Prob	Poss	Neg or other species	No BS and RDT
Pv	831*	0	1 (0.1%)	2 (0.2%)	7 (0.8%)	2 (0.2%)	8 (1%)	6 (0.7%)	5 (0.6%)	71 (8.5%)	3 (0.4%)	55 (6.6%)	17 (2%)	20 (2.4%)	98 (11.8%)	3 (0.4%)
Pf	458*	1 (0.2%)	0	5 (1%)	0	1 (0.2%)	6 (1.3%)	0	32 (7%)	7 (1.5%)	1 (0.2%)	15 (3.2%)	0	44 (9.6%)	32 (7%)	1 (0.2%)

* Are included only infections confirmed by both RDT and BS + the inclusion criteria of the study: no positive RDT in the past 2 weeks, no fever episode in the past 4 weeks, treatment with AL and no critical illness

Definite = BS and RDT positive, Probable = BS positive (RDT neg or absent), Possible = RDT positive (BS neg or absent), Negative = BS and RDT neg

<u>Figure 4-4</u>: Re-attendance within 28 days following an initial positive RDT result

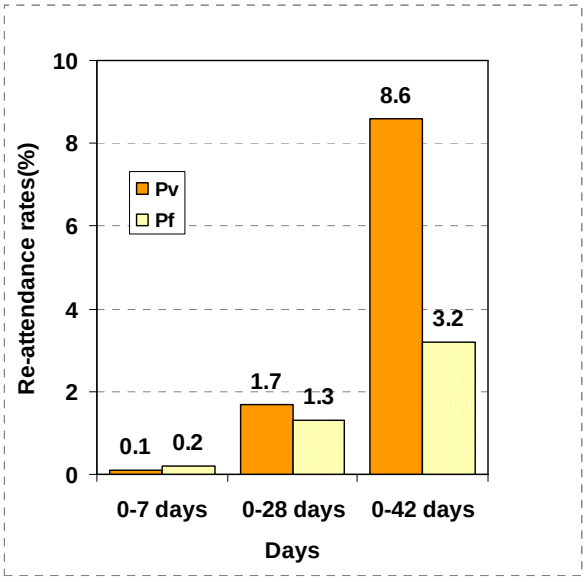


Re-attendance within 7, 28, 42 and 63 days following Pv and Pf infections

Table 4.5 and **figure 4.5** detail the rates of re-attendance following *Pv* and *Pf* malaria episodes confirmed by BS and RDT (definite cases) and treated with AL.

Only re-attendance with the same species is considered, which gives a proxy for clinical treatment effectiveness at 7, 28 and 42 days. 831 definite infections with *Pv* and 458 with *Pf* were included in the analysis. When considering all malaria episodes confirmed by blood slide upon re-attendance (at least the BS is positive), the rate of re-attendance with *Pv* was 0.1% within 7 days, 1.7% within 28 days, 8.6% within 42 days and 12.2% within 63 days. Comparatively the rates for Pf were: 0.2%, 1.3%, 3.2% and 5.7%.

Figure 4-5: re-attendance rates with positive BS within 7, 28 and 42 days for each species (clinical treatment failure rate)



Potential effect of IPTi treatment on rates of re-attendance

No difference was observed in the rates of re-attendance whether the children did receive an IPTi treatment or not in the 6 weeks preceding the fever episode (maximum duration of IPTi post-treatment effect). More specifically, the rate of re-attendance for SAE among 573 children with a negative RDT who came back within 4 weeks for fever was similar in those who got an IPTi dose in the past 6 weeks with 7.7% (34/444) compared to 10% (13/129) in those who didn't (p=0.38).

Finally, no serious adverse event that could be related to AL as assessed by research and routine health staff was observed.

4.5 Discussion

We investigated the safety of managing febrile infants with antimalarials based on results of RDT. The combination of screening for malaria using RDT, and standard treatment of malaria parasitemia with ACT proved to be safe and effective in areas with a high endemicity for both *Pf* and *Pv*.

Although this study was conducted alongside a randomized controlled trial, no active follow-up of study participants was performed and only spontaneous attendance to one of the study clinics or patrols was used to record illness episodes. Such passive case detection may reflect health seeking behavior in routine practice in a setting such as PNG.

Almost 6000 febrile episodes were included in our analysis and one third of them were associated with malaria parasitemia according to the RDT results. No child died or had an SAE because he/she was not treated with artemether-lumefantrine when the RDT was negative. Among the patients with a negative RDT, less than 4% re-attended one of the study clinics within 7 days with persistent fever and only 0.6% (23) were admitted. Less than 1% of children with an initial negative RDT re-attended within 7 days with parasitemia identified by RDT or BS (none had definite malaria based on a positive RDT result and BS). This represents the most conservative estimate of possibly missed malaria cases. Indeed, this includes not only recrudescence and relapses, but also new infections and coincidental parasitemia (most of them had clear alternative diagnosis). Two were admitted with a possibly missed diagnosis of malaria one day after the initial visit (two low densities *Pv on BS,* one with RDT negative result and one without RDT result) during the initial outpatient visit. All these illness episodes were treated appropriately and recovered without sequelae. It is however not possible to tell whether these two cases were truly missed, or if they

experienced a new occurrence of malaria, or whether it was coincidental infection not responsible for the febrile episode.

One child died presumably of a severe pneumonia (malaria was ruled out by RDT and microscopy). These figures provide good evidence that withholding antimalarial drugs from children below 2 years with a negative RDT result is a safe strategy. Indeed, most of the children experiencing an SAE within 7 days following a negative RDT had been already diagnosed with a LRTI during the first visit, but as their conditions worsened, admission was required.

These data are especially reassuring since RDT are known to lack sensitivity for *Pv* infections.(WHO 2008) Fortunately, new generation tests are becoming available with sensitivities for *Pv* as high as those for *Pf.*(Maltha, Gillet et al. 2010) These findings provide for the first time evidence of the safety of withholding antimalarial drugs in infants in a setting highly endemic for *Pv* based on the results of RDT only. They also confirm those of another study performed in Africa where *Pf* is the predominant species(d'Acremont, Malila et al. 2010).

About 1800 malaria cases were diagnosed based solely on the RDT results. All patients of the cohort diagnosed with malaria were treated with artemether-lumefantrine. At the time of the trial, this ACT was not used in PNG and the first line treatment was a combination of sulfadoxine-pyrimethamine and amodiaquine. Therefore we had limited data in the country on the effectiveness and s*afety of this treatment. The present work explores the outcome of children diagnosed with malaria using an RDT and treated with artemether-lumefantrine in routine practice. We found that the rate of re-attendance for fever within 7 days following a positive RDT was very low (less than 2%). This suggests that artemether/lumefantrine was effective at clearing the initial parasitaemia. In the 28 days following initial diagnosis the rate of re-attendance was 10%, but less than 1% of children experienced SAEs, and only 4% of these re-attending children had malaria parasitemia.

Because *Pv* infection can be followed by relapses from hepatic hypnozoites in addition to the recrudescences and re-infections that can occur with *Pf*, we

compared the rates of re-attendances in children with *Pv* and *Pf*. Only 1.7% of children who were initially diagnosed with *Pv* came back with a BS positive for *Pv* within 28 days, while this rate was 1.3% for *Pf* species. By day 42, we found re-attendance rates of 8.2% for *Pv* and 3.2% for *Pf*. These figures do not take into consideration illness episodes where only the RDT was positive upon re-attendance, as it can remain positive weeks after a treatment for malaria because of the circulating. Similarly to re-attendance rates for children with a negative RDT result, these findings are conservative estimates as they include relapses, recrudescences, new infections and coincidental infections. These re-attendance rates are lower than those previously reported in PNG, Indonesia or Africa (Mukhtar, Gadalla et al. 2007; Ratcliff, Siswantoro et al. 2007; Karunajeewa, Mueller et al. 2008; Bell, Wootton et al. 2009; Schoepflin, Lin et al. 2010), This is certainly due, at least partly, to the fact that these studies were designed to assess efficacy of AL rather effectiveness and included therefore systematic screening of parasitemia on defined days rather than spontaneous attendance with illness. The present findings provide unique information on the effectiveness of this combination in real conditions of use, as well as on its safety. The effectiveness of AL against late clinical failures may be further improved by adding a single dose of primaquine to the regimen, as was recently shown in a study in Myanmar(Smithuis, Kyaw et al. 2010). These data indicate that a unified antimalarial treatment policy of AL for both *Pf* and *Pv* is a reasonable option in a setting where resistance of *Pv* to chloroquine is high(Douglas, NM. et al. 2010).(Marfurt, Mueller et al. 2007)

Previous studies in Africa showed that clinicians did not always treat children based on malaria test, results and tended to give antimalarials even to children with negative results.(Reyburn, Mbakilwa et al. 2007; Bisoffi, Sirima et al. 2009)More recent pilot programs of RDT implementation in Zanzibar and Tanzania showed an excellent adherence to a policy of prescription based on positive test results. This strategy led to a dramatic reduction of antimalarial consumption(Williams, Causer et al. 2008; Msellem, Martensson et al. 2009). All these studies were quite heterogeneous in terms of setting and design which

may explain the different results. One of the main contributors to the success in the most recent studies was a comprehensive training program, which led to a true change in clinicians' behavior. The very low rate of AL administration in children with a negative test result in the present trial is also probably the consequence of proper training. Certainly, the research context also helped the clinicians to follow the recommendation of antimalarial treatment based on documented diagnosis.

One of the limitations of our study is the use of a passive case detection system which leaves patients free to attend or not the health facilities when sick. This type of surveillance may miss some malaria episodes, especially when many participants live quite far from the health facility. Also, patients may attend another health facility than that of the study when sick. This should have been a rare event in our study since the clinics were usually the closest facility and care was free of charge for the study children. Some children may have also received from time to time non-prescribed antimalarial drugs or so-called 'over the counter medication' in case of fever for example. However, treatments delivered outside the health system are not widely available in the study areas. In spite of these methodological issues, we can be sure that none of the children died from, or was disabled by malaria, since all children were followed up after their illness episode

In conclusion, this study provides solid evidence that the management strategy for febrile infants in rural outpatient clinics including a screening for malaria using RDT and unified treatment with ACT for malaria infected children is feasible and safe in a setting of high endemicity for *Pf* and *Pv*.

5 Protective efficacy of intermittent preventive treatment in Papua New Guinean infants exposed to *Plasmodium falciparum* and *P. vivax*: a randomized, placebo-controlled trial

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5.1 Abstract

Background

Intermittent Preventive Treatment of malaria in infants (IPTi) is one of the most promising preventative interventions to reduce the burden of malaria in one of the highest risk groups in endemic countries. The concept is to deliver antimalarial treatment to infants at regular intervals, alongside the expanded program of immunization, regardless of the parasitemia. This intervention has been exclusively investigated in Africa where *Plasmodium falciparum* (*Pf*) is the predominant species. The potential benefits of this intervention in areas with significant levels of non-*Pf* infections (mainly *Pv*) is not known.

Methods

Between June 2006 and June 2010 we conducted a 3 arm randomized controlled trial (part of the IPTi consortium) to investigate the efficacy of IPTi with sulfadoxine/pyrimethamine (SP, single dose) associated to 3 days of either amodiaquine (SP-AQ3) or artesunate (SP-AS3) compared to placebo in Papua New Guinea (PNG), a country highly endemic for both *Pf* and *Pv*.

Results: 1125 infants 3 months old were enrolled in the IPTi trial. 4 children were excluded form the analysis because they did not take any of the study drugs. The intention-to-treat (ITT) relative risk (RR) 3-15 months of age was 0.72 (95%CI, 0.57 - 0.90) on all malaria episodes with SP-AQ3 and 0.88 (95%CI, 0.70 - 1.10) with SP-AS3, overall p=0.017. Using SP-AQ3, the RR was 0.63 (95%CI, 0.45 - 0.88) on *Pf* and 0.78 (95%CI, 0.60 - 1.01) on *Pv*. In as-to-protocol (ATP) analysis for SP-AQ3, the RR was 0.49 (95%CI, 0.34 - 0.71) for *Pf* and 0.69 (95%CI, 0.52-0.91) for *Pv*. The RR for anemia with SP-AQ3 was 0.75 (95%CI, 0.58 - 1.01) in the ITT analysis and 0.59 (95%CI, 0.42 - 0.82) in the ATP analysis. No rebound effect was observed during the second year of life. Few deaths were observed in the treatment arms compared to placebo: placebo=8, SP-AQ3=1 and SP-AS3=3. No serious adverse events related to study drugs were observed.

Conclusion

This study established the first evidence on the efficacy of IPTi for the prevention of malaria and anemia in a region highly endemic for both *Pf* and *Pv* and provides an essential proof-of-principal that IPTi is an appropriate strategy for the prevention of *Pv* malaria, if an effective, long half-life drug is used such as SP-AQ3. Policy makers should therefore consider this intervention in areas outside Africa where the burden of non-*Pf* infections is important. Given the levels of resistance to SP and AQ in many parts of the Asia-Pacific and America, further studies are needed to investigate other combinations with long acting drugs with a better efficacy in particular against *P. vivax*.

5.2 Introduction

Malaria and anemia are major causes of morbidity and mortality in children in tropical countries(WHO 2010). Several preventive strategies have proven to be efficient at reducing this burden such as insecticide-treated bed nets(Lengeler 2004)indoor residual spraying(Pluess, Tanser et al. 2010) or prompt diagnosis and treatment with artemisinin combination treatments(Sinclair, Zani et al. 2009; WHO 2010). Additionally, the preventative administration of a full treatment course of antimalarial drugs at fix intervals regardless of illness episodes, named intermittent preventive treatment (IPT), has shown to reduce the morbidity related to malaria in different contexts(Greenwood 2004; Gosling, Cairns et al. 2010 May). This strategy is already widely used in pregnant women (IPTp), with a positive effect on delivery outcomes (Rogerson, Chaluluka et al. 2000; Briand, Cottrell et al. 2007; Crawley, Hill et al. 2007; Menendez, D'Alessandro et al. 2007). More recently, this kind of intervention has shown to be efficient when given to infants at time of vaccination (IPTi) or to older children in areas where malaria transmission is highly seasonal (IPTs)(Cisse, Sokhna et al. 2006; Greenwood 2006; Munday 2007; Clarke, Jukes et al. 2008; IOM 2008; Kweku, Liu et al. 2008; Meremikwu, Donegan et al. 2008; WHO 2008; Aponte, Schellenberg et al. 2009).

In the past 10 years, eight randomized control trials have investigated IPTi in different African countries with various drug regimens. Sulfadoxine-pyrimethamine (SP) dispensed as a single dose 3 or 4 times during the first year of life is the most studied drug and has shown to have a protective efficacy against clinical malaria episodes of 30% (ranging from 20% to 59%). It also reduces the risk of anemia by 21%(Aponte, Schellenberg et al. 2009). In all but one trial the preventative effect of the IPTi dose was restricted almost exclusively to the first 5 weeks after an individual IPTi dose with little effect thereafter(Aponte, Schellenberg et al. 2009). While IPTi with SP retains efficacy in areas with moderate resistance(Mayor 2008; Griffin, Cairns et al. 2010), in areas with very high resistance levels, SP appears to fail to prevent malaria(Gesase, Gosling et al. 2009; Gosling, Gesase et al. 2009). Based on this evidence WHO is now recommending IPTi as suitable malaria control

intervention in areas of high malaria burden and low to moderate SP resistance (WHO 2010).

Two recent clinical trials have investigated alternative drug regimens that included as least 1 long-half life antimalarial such as mefloquine (125 mg single dose), a combination of SP (stat) plus 3 days of artesunate (AS) or amodiaquine plus AS (3d) for the use as IPTi. All these treatments have protective efficacies ranging from 26-38%(Gosling, Gesase et al. 2009; Odhiambo, Hamel et al. 2010) and were comparable to those in earlier SP trials. While mefloquine showed the highest effect (38%), the tolerability of mefloquine was poor with 8% of the children vomiting the drug after its administration. A shorter acting drug, dapsone/chlorproguanil however failed to significantly reduce both the burden of malaria and anamiea (Gosling, Gesase et al. 2009; Odhiambo, Hamel et al. 2010). This indicates that IPTi works mainly through a prophylactic effect achieved by long acting drugs (prevention of new infections) rather than through a therapeutic effect (cure of existing parastiemia) (May, Adjei et al. 2008).

All IPTi studies have been exclusively carried out in Africa where *Plasmodium falciparum* (*Pf*) is the predominant parasites and *P. vivax (Pv)* is virtually absent due to the high prevalence of Duffy negativity (Rosenberg 2007). In highly endemic areas of the SW Pacific,(Genton, D'Acremont et al. 2008; Lin, Kiniboro et al. 2010) SE Asia (Tjitra, Anstey et al. 2008) and the Americas (Alexandre, Ferreira et al. 2010), *Pv* is however a major source of morbidity including severe illness and death in particular in young children. An intervention specifically targeting infants like IPTi may thus have significant benefits in these areas. Unfortunately, to-date, nothing is known about IPTi efficacy in non-African settings with a high burden of non-*Pf* infections, where IPTi might be considered as part of national malaria control strategies. African results cannot be easily extrapolated to *Pv* co-endemic areas both due the difference in the biology of *Pf* and *Pv*, in particular its ability to relapse from long-lasting liver-stages and the ability of *Pv* to quickly acquired resistance to SP (Tjitra, Anstey et al. 2008).

In order to determine the efficacy of IPTi in reducing the burden of malaria and anaemia in a *Pf* and *Pv* co-endemic setting we therefore undertook an randomized, placebo-controlled trial of IPTi with two different drug regimens combining a long acting drug (SP) with either 3 days of another drug with long half-life (AQ) or 3 days of a short acting drug (AS) in an area of Papua New Guinea (PNG) that is a highly endemic country for both *Pf* and *Pv.*

5.3 Methods

Trial design, sites and study participants

We undertook an individually randomized, double blind, placebo-controlled trial of two different regimes (sulfadoxine/pyrimethamine (stat) with either 3 days of amodiaquine (SP-AQ3) or 3 days of artesunate (SP-AS3)) of IPTi. The study took place from the 6th of June 2006 to the 16th of June 2010. All drugs were given at routine vaccination time points at 3, 6 & 9 months and Vitamin A supplementation at 12 months following the PNG national expanded program of immunization (EPI).The efficacy of the intervention was assessed for a 12 months period from enrolment to a primary assessment time point at 15 months of age. Afterwards children continued to be monitored every 3 months for up to 12 additional months (see table 1)

The study was carried out on the north coast of mainland Papua New Guinea (PNG) in the Mugil area of Sumkar District, Madang Province. The study site included 20 villages situated in an approximate 400 km^2 area between the coastline and the foothills (<400 m) of Adelbert Range, 30-60 km north of Madang town. The Madang north coast receives an ample amount of rainfall all year round (3000-4000mm / year) with a short dry season from June to September. The 20 study villages are serviced by 2 major health centres (Mugil and Alexishafen) and several aid posts. Both Health centers are responsible to deliver the Expended Program of Immunization (EPI) program in surrounding villages through monthly outreach clinics.

Free distribution of long-lasting insecticide treated nets (ITN) by the national malaria program only started at the end of 2008. Until then, a majority of the population in the study site was using untreated nets.

Originally, the study was started as a two-site study with a second site in Maprik (East Sepik Province). However, an interim analysis performed at mid-study found that the incidence of malaria was dramatically different between the two sites (approximately 0.2 episode/child/year for both *Pf* and *Pv* in Maprik compared to 1.0 in Mugil) restricting the comparability of the two sites. Consequently, the study was terminated in Maprik (enrolments ceasing in May 2008). Data from the Maprik cohort was only included in the safety analyses. The Data and Safety Monitoring Board (DSMB) was informed and agreed on the changes made.

The study was carried out in accordance with Good Clinical Practice (GCP) guidelines as required by the International Conference on Harmonization (ICH) GCP E6 and monitored by an independent external monitor. An independent DSMB composed of 2 clinicians and one statistician was also constituted. The study was approved by the PNG Medical Research Advisory Committee (MRAC number 05.20). The trial was registered on www.clinicaltrials.gov (number NCT00285662) and formed part of the IPTi consortium (www.ipti-malaria.org) (Schellenberg, Cisse et al. 2006).

Randomization, blinding and treatment allocation

The inclusion criteria were: 1) permanent resident of the area, 2) aged 2-4 months old, 3) no disability, 4) no chronic illness, 5) no allergy to study drugs, 6) Hb>5g/l and 7) weight for age > 60%. The children meeting these criteria were randomly allocated to receive placebo, SP-AQ3 or SP-AS3 treatment group using a pre-assigned list. In order to assure blinding of the trial, either treatments or placebo were randomly assigned to 4 different treatment codes each, resulting in a total of 12 treatment codes. The randomization list was produced by an independent statistician and held by the DSMB. Opaque envelops with consecutive ID containing

allocated treatment codes were prepared in advance for the field staff. Only when a child met the inclusion criteria, he/she was allocated to the next available ID number and the corresponding treatment code by opening the next envelope.

AS & SP or placebo (IPCA Ltd, India) and AQ or placebo (Kimapharma, Ghana) were pre-packaged in individual blisters. Study drugs and placebo were assigned to the respective treatment codes by an independent technician using the allocation list produced by the independent statistician. Quality of drugs and correct code allocation was cross-checked by testing drug content in a random sample of tablets at the school of Medicine & Pharmacology, University of Western Australia.

Clinical Study Procedures

The three different treatment regimens were a single dose of SP (25mg sulfadoxine and 1.25mg pyrimethamine/kg) combined with 3 days of AQ at 10mg per kg (SP-AQ3) plus 3 days of AS placebo; a single dose of SP plus 3 days of AS at 4mg per kg (SP-AS3) plus 3 days of AQ placebo or matching placebos for all three drugs. The tablets were split (quarters or halves) according to the weight (kg) of each child. These two treatments arms correspond to the first and second lines of treatment of the PNG national standard guidelines for malaria until 2011 when artemether/lumefantrine (AL) will be adopted as first and DHA-piperaquine as 2nd line treatment.

The intervention was delivered four times during the first year of life alongside the Expanded Program of Immunization (EPI). The first IPTi treatment dose was delivered at enrollment at 3 months of age along with the third dose of immunization against poliomyelitis (OPV), hepatitis B (HBV), diphtheria/pertussis/tetanos (DPT) and *Haemophilus influenza* type b (Hib, introduced in 2007).The second IPTi dose was administered at 6 months of age along with first doses of measles vaccine and vitamin A. The third IPTi dose was given at 9 months of age along with second dose of measles vaccine. Finally the fourth and last IPTi dose was delivered at 12 months of age along with the second dose of vitamin A.

The drugs were crushed and mixed with water and sweet syrup (Golden Crush Cordial ® Papua New Guinea) for easy administration with spoon or syringe. The first day dose was administered by a study staff during the clinic. The child was monitored closely and if he/she vomited within the next 30 minutes the dose was repeated. The 2nd and 3rd doses were given by the carer at home without direct supervision. To assess adherence and potential drug adverse reactions, a community reporter in each village was tasked to visit each child every day that a study treatment dose was given.

Children were recruited into the IPTi study alongside monthly outreach maternal and child health clinics (MCH), which were run by specifically trained study nurses jointly with medical staff of both health centres under the supervision of two study physicians.

Enrollment of study participants

The parents or guardians of potential study participants were contacted during routine EPIclinics when the child was 1-2 months of age. The study was explained in details to the parents through both individual and community awareness meetings and an information brochure and consent form in English and in the local language (Pidgin) was given to the parent to take home for further discussion.

The child who met the inclusion criteria was formally enrolled during the next clinic when the child was 3 months of age (more or less 1 month) and after parents gave their written consent

In total, up to 9 visits were scheduled. Upon enrollment at 3 months of age, a concise medical history including bed net use, possible disabilities and presence of acute illness was performed. A brief physical examination was done including measurement of weight (with weight for age reporting) and spleen size. **Table 5.1** summarizes the different visits performed all along the trial.

Table 5-1: Study schedule of the IPTi trial with immunizations and blood collection time points

Type of Visit	Age (months)									
	2	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**
Pre-screening										
IPTi treatment		IPTi 1	IPTi 2	IPTi 3	IPTi 4					
Primary efficacy assessment										
Follow-up										
Final assessment										
Passive case detection										
Immunization	OPV3	OPV4								
		HBV3								
	DPT2	DPT3								
			Meas 1	Meas 2						
			Vit A		Vit A					
During schedulded visits										
blood collection from finger prick		500µl	250µl	250µl	1 ml	250µl	250µl	250µl	250µl	1ml
Hb level & spleen measurment										

Throughout the study period, a passive case detection system was maintained at the Mugil study health centre outpatient clinic as well as at three aid posts within the study area to assure a continuous morbidity surveillance of study participants. The parents of study participants were encouraged to attend these study clinics free of charge for clinical management whenever their child was sick. Each illness episodes was assessed by study staff using a standard case report form. In case of history of fever in the past 48 hours or an axillary temperature > 37.5°, rapid malaria diagnostic tests (RDT, ICT Combo®, South Africa) was performed, two blood smears and 250 microliters of whole blood collected and Hb level measured using a portable Hemocue 201® machine (Angelholm, Sweden). Treatment decision was based solely on RDT results with only RDT positive infants treated with artemether/lumefantrine (Coartem®), irrespective of the species. The children with moderate to severe anemia (Hb < 8g/dl) received iron supplementation at the dose of 5mg/Kg of ferrous sulphate for 6 weeks. All other illnesses were treated according to the standard treatment guidelines of PNG. Children presenting any danger signs or symptoms were referred to the health centre for admission and treatment. If study participants attended after hours, they were attended to by the health centre nurses directly. In this case, study staff recorded the reason of admission with clinical presentation and treatment allocation (when ever available) the next day. A finger prick could be done if necessary to check for malaria parasites and Hb levels. Usually, the health center staff did not have the capacity to perform any blood test such as malaria rapid diagnostic test at the time of admission and treatments were delivered on presumptive diagnosis.

All illness episodes were considered as adverse events and were graded by the study clinicians according to the severity from 1 (less severe) to 3 and serious adverse event (SAE: life-threatening or death). Hospital admissions that did not fulfill the criteria of severity were considered as grade 3. Causality of AE was also assessed by the study clinicians as: not related, unlikely, possible and certain. All SAE and AE possibly or certainly related were reported to the DSMB. Grade and causality were assessed using guidelines adopted by the IPTi Consortium.

Laboratory procedure

Malaria diagnosis

All blood slides were read independently by two expert microscopists. In case of discrepancies, a third read was performed by another microscopist and the two concordant reads of the three were considered as the final result. Thick blood films were examined by light microscopy (LM) for 100 thick-film fields (under 100X oil immersion lens) before being declared infection-negative. Parasite species in positive films were identified and densities recorded as the number of parasites per 200 white blood cells (WBC). Densities were converted to the number of parasites per ml of blood assuming 8,000 WBC per ml (population average WBC count [3]). Slides were scored as LM-positive for an individual *Plasmodium* species, if the species was detected independently by at least two microscopists. Parasites densities were obtained by calculating the geometric mean between the two reads. A third read was performed if the first two slides readings were discrepant (different species or more than one logarithm difference in parasites densities).Additionally, if the final slides readings (after 2 or 3 reads) were discrepant compared to PCR results(positivity and species), they underwent a $3^{rd}/4^{th}$ read by a senior microscopist and this last read was kept as the final BS result.

Blood samples were collected from finger pricks in tubes coated with Ethylenediaminetetraacetic acid (EDTA) and/or in plain tubes to obtain serum depending on the visit. Upon arrival in the laboratory, samples were separate into plasma/serum and cell pellet. Plasma and serum were stored at -80°C until further use. DNA was extracted from cell pellets using the QiaAMP 96 extraction kit (Qiagen) and the 96-well Genomic DNA Extraction Kit (Favorgen).

The presence of each of the four human malaria species was also assessed in all blood samples using a semi-quantitative post-PCR, ligase detection reaction/fluorescent microsphere assay (LDRFMA) ((McNamara, Kasehagen et al. 2006))). This assay combines PCR amplification of the small subunit (ssu) ribosomal RNA gene (491–500 bp fragments) using genus specific primers, followed by a multiplex species-specific ligation detection reaction (LDR). LDR products are then incubated with a solution of streptavidin-phycoerythrin and specific fluorescent microspheres. The fluorescent signals are collected through

a Bioplex reader (Bio-Rad®). The design and sensitivity of this assay has been described previously. Mix of serial dilutions of clones of *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* 18s RNA fragments were used as controls of reactions, as well as two pools of *P. falciparum* or *P. vivax* positive samples obtained from field isolates. The threshold of positivity of the reactions for each specie was determined monthly using the mean value obtained for the negative controls (for each specie), plus 3 times the value of its standard deviation. Samples with low-positive values for one specie (< threshold + 5 times the standard deviation) were double-checked and considered as positive only if the result was confirmed.

Molecular marker of drug resistance typing

A subset of asymptomatic infections with Pf or Pv were typed for molecular markers of antimalarial resistance using two post-PCR LDRFMA. Markers of Pf SP drug resistance included mutations in *pfdhfr* (dihydrofolate reductase gene – codons 51, 59, 118, 164) and *pfdhps* (dihydropterate synthase gene – codons 436, 437, 540, 581, 613); markers of Pf 4-aminoquinoleine drug resistance included mutations in *pfcrt* (chloroquine transporter gene – codons 72 to 76) and *pfmdr1* (multidrug resistance 1 gene – codons 86, 184, 1034, 1042, 1286). The protocol published by Carnevale *et al* (Carnevale, Kouri et al. 2007) was optimized towards a simultaneous amplification of five fragments of these genes (*pfdhfr, pfdhps, pfcrt* and two fragments of *pfmdr1*) in a multiplex nested PCR, with newly designed primers for the five gene fragments (see additional files for sequences). A single LDR was performed with 32 allele-specific primers (see additional files for sequences). Threshold of positivity Markers of Pv SP drug resistance included mutations in *pvdhfr* (codon 57 to 61, 117, 173), *pvdhps* (codons 382-383, 553, 647) and to 4-aminoquinoleines, *pvmdr1* (codon 976).The analysis was conducted as described elsewhere.

Sample size calculations

Based on a predicated incidence rate of 1.2 episodes / child/year (0.7 for *Pf* and 0.5 for *Pv*), a power of 90% and alpha = 0.025, a sample size of 250 children per arm was estimated to be necessary to find at least a 28% reduction in the incidence of all malaria episodes, 30% reduction in incidence of *Pv* episodes and 35-37% reduction in incidence of *Pf*. Assuming a drop out of participants of 20%

in conjunction with a fall in incidence of malaria of 30 to 50% due to insecticide-treated bed nets (ITN) introduction through the national program in November 2008, a final adjusted samples size of 366 children per arm or 1100 children in total has been calculated.

Statistical methods

The analysis was performed both by Intention to treat (ITT) and also according to protocol (ATP). The ITT population includes all randomized children in Mugil. Following the intention-to-treat principle, subjects were analyzed according to the preventive treatment they were assigned to at randomization. In the ATP analysis, the population includes all randomized subjects that received at least 3 IPTi treatments (each given with 4 weeks after the schedules treatment time point) and were under passive surveillance for the entire 12 months period.

The primary objective of the trial was determination of protective effect of the two IPTi regimens. The primary outcome was measured as the incidence of symptomatic malariadue to any Plasmodium species from 3 - 15 months of age. Secondary outcomes included 1) the specific incidences of symptomatic malaria due to Pf or *Pv* from 3 to 15 months of age, 2) the incidence of symptomatic malaria (any, *Pf* and *Pv*) from 15 - 27 months (i.e. 'rebound'), 3) the incidence of moderate-to-severe (Hb < 8 g/dl) and severe anaemia (Hb<5 g/dl) from 3 to 15 months and 15 to 21 months, 4) the prevalence and density of malaria parasitemia at 15 and 21 months and 5) the prevalence of splenomegaly at 15 and 27 months.

Symptomatic malaria was defined as (history of fever and/or axillary temperature >37.5° and a positive blood smear or a positive RDTm, confirmed by positive PCR). The incidences between the groups are compared using poisson regression models with gamma random effects at the intercept (negative binomial regression) to take into account possible extra-poisson variation due to frailty at individual level. Analysis were adjusted by sex, genotype, number of IPTi treatments received, season of enrolment (wet vs dry) and site (grouped by 12 recruitment zones). The time at risk was calculated starting the date of enrollment until the date defined according to the analysis, or withdrawn from the

study. An arbitrary period of 28 days was excluded after each episode. The same methodology was used for the evaluation of the incidence of moderate-to-severe (Hb < 8 g/dl) and severe anemia (Hb<5 g/dl). For the analysis of Hb concentration, the mean and standard deviation are presented by group. Difference between groups was evaluated using Ordinary Least Square Regression, adjusting by sex, genotype, number of IPTi treatments received, season of enrolment and site.

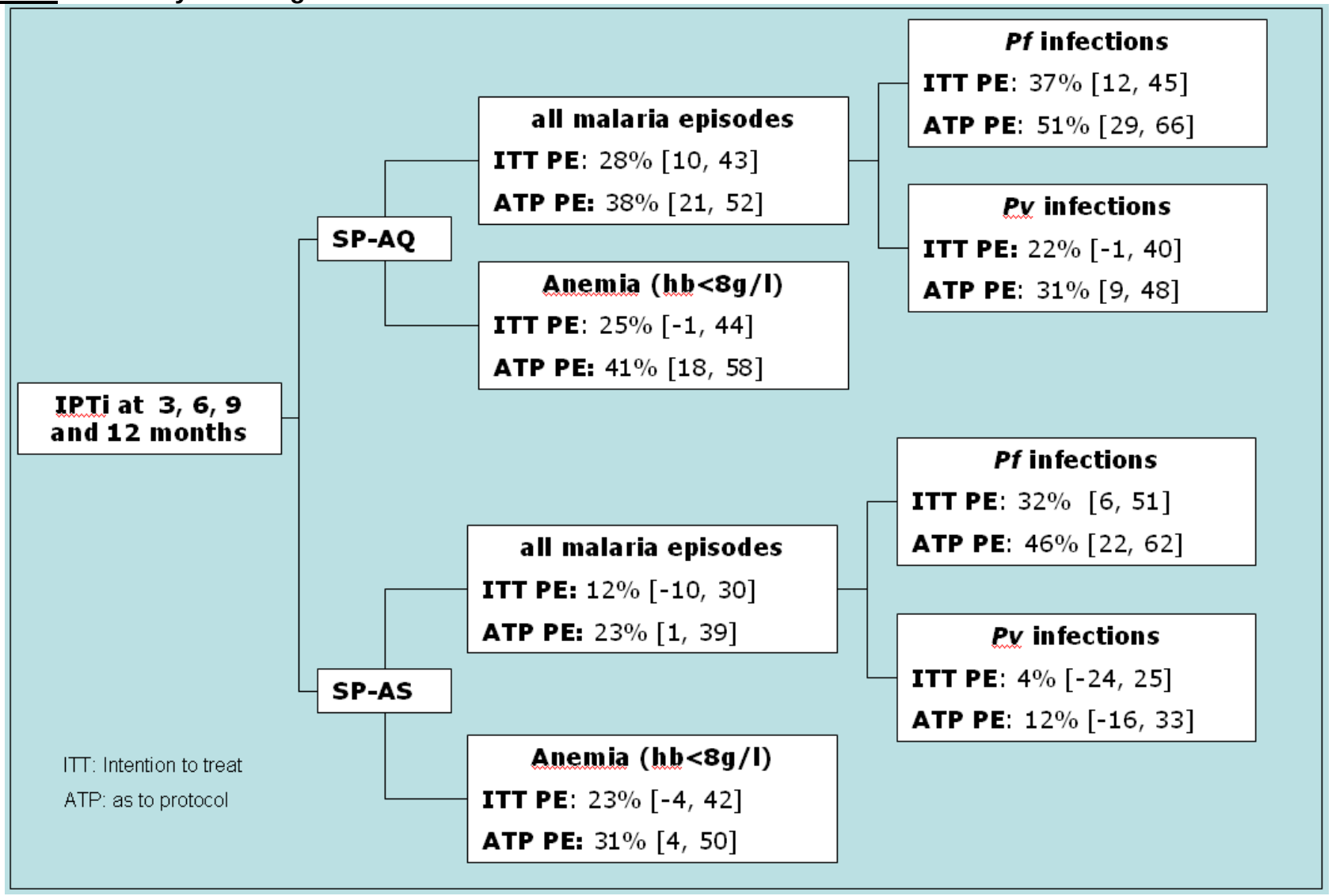
For the analysis of prevalence of anemia and splenomegaly, the number of subjects that accomplish the definition, percentage and the population at risk is presented by group. Comparison between groups was analyzed using logistic regression adjusting by sex, genotype, number of IPTi treatments received, season of enrolment and site. The risk of malaria in the 35 days following the administration of each of the treatment dose was investigated by calculating incidence rates and hazard ratios using a cox regression model.
The analyses were performed in Stata version 11. (Stata statistical software, College station, TX, USA).

Role of the founding source
The sponsor of the study has no role in the design and conduct of the study. All collected data are stored at the PNG IMR in Madang.

5.4 Results

From June 2006 to June 2010, 1125 infants 3 months old were enrolled, randomized and followed-up in the trial in Madang area (**Figure 5.1**). Four were retrospectively excluded from the analysis as they were already receiving antimalarial treatments at the time of all IPTi visits and therefore didn't receive any IPTi treatment dose. 374 were allocated to SP-AQ3 group, 374 to SP-AS3 group and 373 to placebo group (Figure 1). A total of 1079 (96%) completed follow-up to 15 months, whereas 857 (76%) completed the primary 6 months and 372 (33%) the extended 12 months post-treatment follow-up. At the Maprik site, 480 children were enrolled from July 2006 to May 2008.

Figure 5-1: **IPTi Study flow diagram**



The baseline characteristics of all Madang study infants were similar across the 3 treatment arms (**Table 5.2**). No significant imbalances were observed between the 3 groups. In the final multivariate models gender, village of residence, ITN use in the past 2 weeks and season of recruitment were included.A total of 939 children received 3 or 4 IPTi treatments were thus included in the ATP population with no significant imbalances between groups.

Table 5-2: Baseline Characteristics of the study participants

			PLACEBO	AQ-SP	AR-SP
N			373	374	374
Girls			192 (51.5%)	166 (44.4%)	187 (50%)
Village areas	Biranis		18 (4.8%)	20 (5.4%)	18 (4.8%)
	Megiar		20 (5.4%)	17 (4.6%)	22 (5.9%)
	Aronis Garup Wasabamal Zizzi		41 (10%)	35 (9.4%)	42 (11.2%)
	Basken Dimer		77 (20.6%)	74 (19.9%)	72 (19.3%)
	Bunu Kusen Mugil		38 (10.2%)	54 (14.4%)	54 (14.4%)
	Matukar Wasab		38 (10.2%)	31 (8.3%)	24 (6.4%)
	Dylup		20 (5.4%)	29 (7.8%)	22 (5.9%)
	Karkum		16 (4.3%)	8 (2.1%)	20 (5.6%)
	Mirap		17 (4.6%)	23 (6.2%)	31 (8.3%)
	Sareng		30 (8%)	21 (5.6%)	23 (6.2%)
	Taldig CCI		41 (11%)	48 (12.8%)	33 (8.8%)
	Rempi		17 (4.6%)	14 (3.7%)	13 (3.5%)
Slept under bednet last two weeks			308 (82.8%)	299 (80%)	305 (82%)
Recruitment during rainy season (September-June)			303 (81.2%)	301 (80.5%)	301 (80.5%)
Mean Age at enrollment (days)			96 (15)	99 (18)	98 (16)
Weight (Kg)			5.9 (0.8)	6.0 (0.9)	6.0 (0.8)
Haemoglobine (g/dl)			9.5 (1.0)	9.5 (1.2)	9.5 (1.1)
Prevalence of parasitemia at baseline (light microscopy)	All species		14 (3.8%)	30 (8%)	24 (6.4%)
	Pf		7 (1.9%)	13 (3.5%)	9 (2.4%)
	Pv		7 (1.9%)	17 (4.5%)	15 (4%)
Red blood cells polymophysms					
	α-thalassemia				
		αα/αα	53 (17.4%)	63 (19.6%)	53 (16.9%)
		αα/α-	134 (44.1%)	134 (41.7%)	143 (45.5%)
		α-/α-	108 (35.5%)	112 (34.9%)	103 (32.8%)
		Unknown	9 (3%)	12 (3.7%)	15 (4.8%)
	Gerbich blood group				
		wt/wt**	171 (56.3%)	192 (59.8%)	201 (64%)
		wt/Δex3	86 (28.3%)	92 (28.7%)	77 (24.5%)
		Δex3/Δex3	35 (11.5%)	24 (7.5%)	26 (8.3%)
		Unknown	12 (4%)	13 (4.1%)	10 (3.2%)
	SAO				
		wt	263 (86.5%)	273 (85.1%)	275 (87.6%)
		sao	34 (11.2%)	44 (13.7%)	30 (9.6%)
		Unknown	7 (2.3%)	4 (1.3%)	9 (2.9%)

Data are means (SD) and n(%)
** wild-type

Protective efficacy (PE)

The incidence of clinical malaria between the first dose at 3mth and 15 mth was 0.85 per year-at-risk overall and 0.22 and 0.68 for *P. falciparum* and *P. vivax,* respectively. In ITT analyses, PE against all cases of malaria was 29% ([95%CI, 0.10, 0.43], p = <0.001) in the SP-AQ3 group and 12% ([95%CI, -0.11, 0.30], p = 0.12) in the SP-AS3 group. The PE was higher against *P. falciparum* than *P. vivax* in both groups *(***Table 5.3 & 5.4**): In the SP-AQ3 group, *Pf* incidence was reduced by 35% ([95%CI, 0.09, 0.54], p = 0.012) and *Pv* incidence by 23%([95%CI, 0.00, 0.41], p = 0.048). In the SP-AS3 group on the other hand a significant reduction was found only for incidence of *P. falciparum* (IRR = 0.69, 95%CI, 0.49, 0.96] p = 0.027) for but not *P. vivax* episodes (IRR = 0.96, [95%CI, 0.94, 1.24] p = 0.758).

The strength of protection increased when only children with ≥3 IPTi treatments were considered (i.e. APT analyses, **Table 5.4**). Regular administration of SP-AQ3 reduced malaria episodes by 38% overall ([95%CI, 0.20, 0.52] p < 0.001) and 49% ([95%CI, 0.26, 0.65] p < 0.001) and 31% ([95%CI, 0.09, 0.49] p = 0.010) for *P. falciparum* and *P. vivax* malaria episodes*,* respectively, whereas administration of SP-AS3 resulted in a 23% reduction in all malaria episodes ([95%CI, 0.01, 0.40] p = 0.041) and a 46% ([95%CI, 0.22, 0.62] p = 0.001) and 12% ([95%CI, -0.12, 0.34] p = 0.355) for *P. falciparum* and *P. vivax* episodes.

Table 5-3: efficacy results of IPTi 3-15 months (ITT)

	Group	Number of cases	Time At Risk (PYAR)	Unadjusted values				Adjusted values			
				Incidence per PYAR	RR	95% Conf. Interval	p-value	Incidence per PYAR	RR	95% Conf. Interval	p-value
All malaria episodes	Placebo	362	337.3	1.07	1	-		1.07	1	-	0.017
	AQ-SP	280	352.1	0.80	0.71	(0.57, 0.90)	0.018	0.80	0.72	(0.57, 0.90)	
	AR-SP	336	346.9	0.97	0.88	(0.70, 1.11)		0.97	0.88	(0.70, 1.10)	
Pf infections	Placebo	100	356.1	0.28	1	-		0.28	1	-	0.012
	AQ-SP	67	366.6	0.18	0.65	(0.46, 0.91)	0.020	0.18	0.63	(0.45, 0.88)	
	AR-SP	71	365.4	0.19	0.69	(0.49, 0.96)		0.19	0.68	(0.49, 0.94)	
Pv infections	Placebo	282	342.9	0.82	1	-		0.82	1	-	0.130
	AQ-SP	234	355.4	0.66	0.77	(0.59, 1.00)	0.108	0.66	0.78	(0.60, 1.01)	
	AR-SP	283	350.5	0.81	0.96	(0.74, 1.24)		0.81	0.96	(0.75, 1.24)	
Anemia (Hb < 8g/dl)	Placebo	169	351.1	0.48	1	-		0.48	1	-	0.110
	AQ-SP	132	362.6	0.36	0.75	(0.56, 1.01)	0.090	0.36	0.75	(0.56, 1.01)	
	AR-SP	133	361.2	0.37	0.75	(0.56, 1.01)		0.37	0.77	(0.58, 1.04)	
Severe malaria	Placebo	12	362.1	0.03	1	-		0.03	1	-	0.906
	AQ-SP	10	370.4	0.03	0.81	(0.33, 1.97)	0.894	0.03	0.82	(0.33, 2.00)	
	AR-SP	11	369.6	0.03	0.89	(0.37, 2.13)		0.03	0.9	(0.38, 2.15)	

p-value from Negative binomial regression model using Likelihood Ratio Test

Treatment effect adjusted by:sex, village areas, slept under bednet last two weeks and season at recruitment

Table 5-4: Efficacy results of IPTi 3-15 months (ATP)

	Group	Number of cases	Time At Risk (PYAR)	Unadjusted values				Adjusted values			
				Incidence per PYAR	RR	95% Conf. Interval	p-value	Incidence per PYAR	RR	95% Conf. Interval	p-value
All malaria	Placebo	302	285.84	1.06	1	-		1.06	1	-	0.0008
	AQ-SP	212	310.45	0.68	0.62	(0.48, 0.80)	0.001	0.68	0.62	(0.48, 0.79)	
	AR-SP	250	300.04	0.83	0.77	(0.60, 0.99)		0.83	0.77	(0.61, 0.99)	
Pf infections	Placebo	83	301.58	0.28	1	-		0.28	1	-	0.0001
	AQ-SP	45	321.71	0.14	0.51	(0.35, 0.74)	0.0002	0.14	0.49	(0.34, 0.71)	
	AR-SP	47	314.14	0.15	0.54	(0.38, 0.78)		0.15	0.54	(0.38, 0.78)	
Pv infections	Placebo	235	290.41	0.81	1	-		0.81	1	-	0.033
	AQ-SP	180	312.79	0.58	0.69	(0.52, 0.91)	0.0325	0.58	0.69	(0.52, 0.91)	
	AR-SP	218	302.32	0.72	0.88	(0.66, 1.16)		0.72	0.88	(0.67, 1.16)	
Anemia (<8g/dl)	Placebo	143	297.21	0.48	1	-		0.48	1	-	0.0048
	AQ-SP	92	318.92	0.29	0.59	(0.42, 0.82)	0.0037	0.29	0.59	(0.42, 0.82)	
	AR-SP	101	310.39	0.33	0.66	(0.47, 0.92)		0.33	0.69	(0.50, 0.96)	
Severs malaria	Placebo	11	306.412	0.036	1	-		0.036	1	-	0.6545
	AQ-SP	7	324.153	0.022	0.6	(0.22, 1.66)	0.6115	0.022	0.62	(0.23, 1.71)	
	AR-SP	9	316.767	0.028	0.79	(0.30, 2.05)		0.028	0.81	(0.32, 2.09)	

p-value from Negative binomial regression model using Likelihood Ratio Test

Treatment effect adjusted by:sex, village areas, slept under bednet last two weeks and season at recruitment

IPTi with both SP-AQ3 and SP-AS3 also provided protection against moderate-to-severe anaemia (Hb < 8 g/dl, **Table 5.3 & 5.4**). In the ITT population, the incidence of anaemia was reduced by 25% for both SP-AQ3 ([95%CI, -1, 44] p = 0.054) and SP-AS3 (([95%CI, -1, 44] p = 0.062). In the ATP population (i.e. ≥3 IPTi treatments) the protective efficacy increased to 41% ([95%CI, 18, 58] p = 0.002) in the SP-AQ3 and 34% ([95%CI, 8, 53] p = 0.013) in the AS-AS3 group. The study shows also a reduction of the risk of moderate-to-severe anemia (hb < 8g/dl) by 25% with SP-AQ3 in the ITT analysis (p=0.11) and 41% in the ATP analysis (p=0.005).

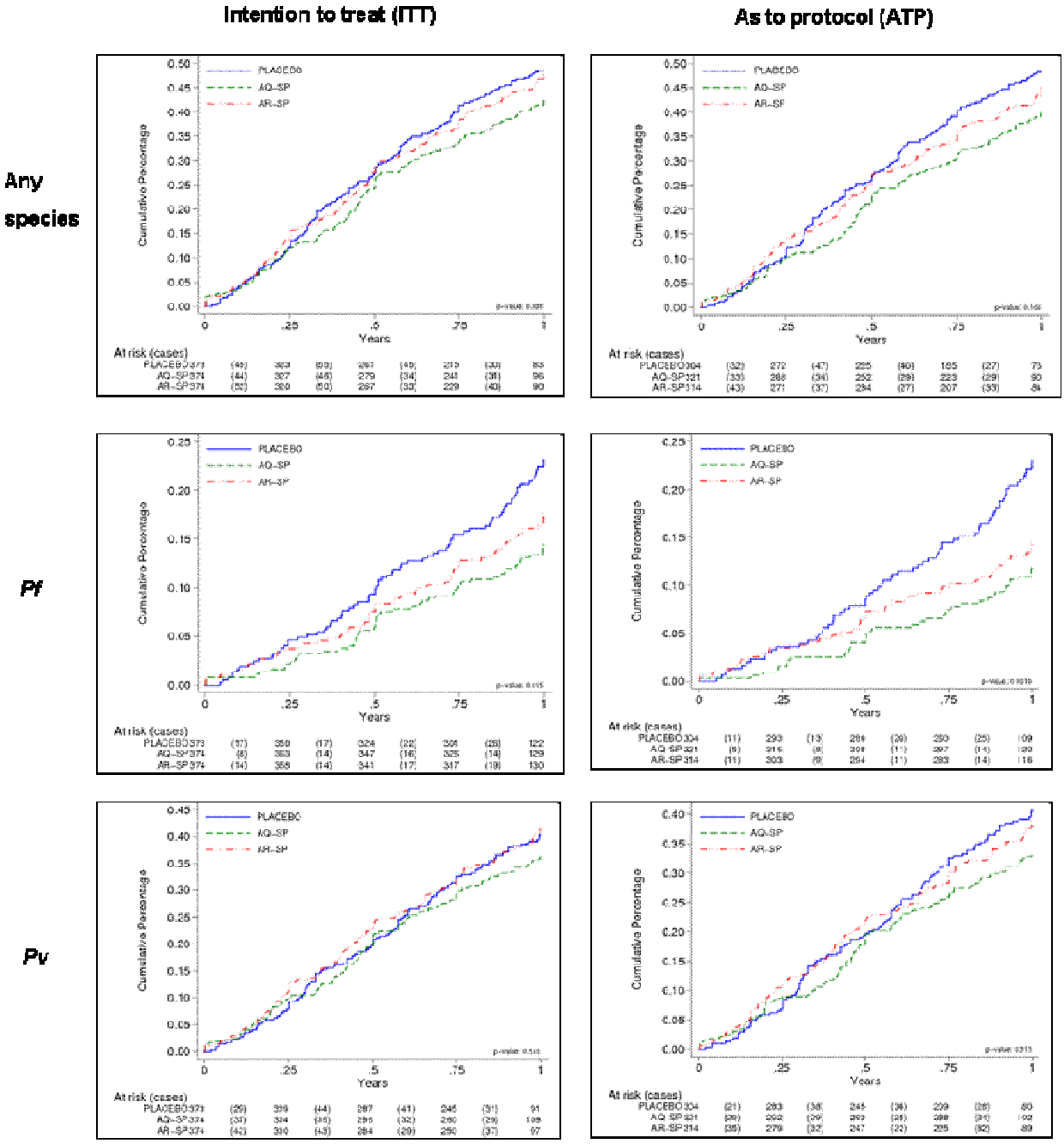
Few cases of severe anemia (< 5g/dl) were observed during the study. In the ITT analysis, there were 8 severe anaemia in the placebo group, 2 in the SP-AQ3 group and 4 in the SP-AS3 group (p=0.12). In the ATP analysis, there were 7 cases in the placebo, 1 in SP-AQ3 group and 2 in the SP-AS3 group (p=0.04).

Adjustment for sex, place of residency, season of recruitment and use of bed nets in the past two weeks did not significantly alter estimates of PE (figure 5.2).

Figure 5-2: Summary of IPTi preventive efficacy of malaria at 15 months of age (all RR were adjusted for sex, place of residence, season of enrolment and use of bed nets in the past 2 weeks)

Graph 5.1 shows the Kaplan-Meier survival curves for the time to the first or only malaria episode 3 to 15 months in ITT and ATP analysis for any species, *Pf* and *Pv* infections. Hazard ratios (HR) were as follow for SP-AQ3 in the ITT analysis: 0.85 [95%CI 0.69, 1.05] for all species, 0.60 [95%CI, 0.42, 0.84] for *Pf* and 0.92 (95%CI, 0.73, 1.16) for *Pv*. In the ATP analysis, HR for SP-AQ3 were 0.79 (95%CI, 0.62, 0.99) for all species, 0.50 (95%CI, 0.34, 0.74) for *Pf* and 0.82 (95%CI,0.63, 1.06) for *Pv*.

<u>Graph 5-1:</u> Kaplan-Meyer survival curves of the IPTi protective efficacy using either SP/AQ or SP/AS on all malaria episodes, on *Pf* and on *Pv*



Post-dose efficacy

SP-AQ3 had slightly lower protective efficacy in the 35 days following the allocation of each IPTi treatment doses against *Pf* (72% [95%CI, 39 – 87], p < 0.001) than *Pv* (83% [95%CI, 70 – 90], p < 0.001), whereas SP-AS3 was more efficacious against *Pf* (82% [95%CI, 53 – 93], p<0.001) than *Pv* (58% [95%CI, 36 – 73], p<0.001). (**Table 5.5**)

Post-intervention risk

The incidence of clinical malaria between 15 and 21 mths was 1.09 per year-at-risk overall and 0.43 and 0.70 for *P. falciparum* and *P. vivax,* respectively. No evidence of a 'rebound effect' was observed in the 6 mths following the end of the intervention at the age of 15 mths (p=0.79 between all three groups in the ITT analysis). Similarly, no significant differences in incidence of *P. falciparum* malaria, *P. vivax* malaria and anaemia were observed (**Table 5.6**). There was also not indication of a rebound effect in the subset of children that were followed up to 27mths (p=0.99 between all 3 groups in the ITT analysis).

Prevalence of parasitaemia during follow-up

The prevalence rates of both *P. falciparum* and *P. vivax* increased from 1.5% and 8.5% at 6mth to 3.0% and 15.8% at 15mth and 4.4% and 20.3% at 21mth, respectively, when detected by microscopy (**Figure 5.3**). At 15 months, the prevalence of *P. falciparum* was significant lower in the two treatment arms (p = 0.037) but not at any other time point. No significant differences in *P. vivax* prevalence were found between treatment arms. Only 2 *P. malariae* and no *P. ovale* infections were detected by light microscopy

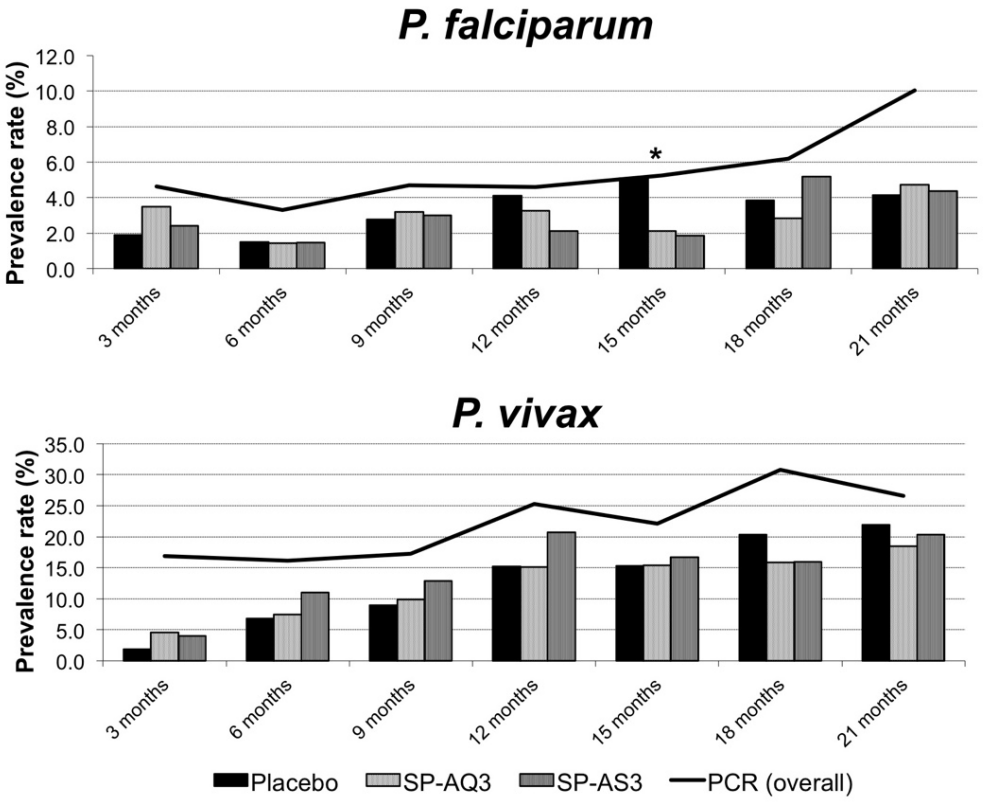
Table 5-5: protective efficacy in the 35 days following each treatment dose

		Group	Numbner of cases	Time At Risk (PYAR)	Incidence per PYAR	HR	95% Conf. Interval	p-value
After dose 1 at 3 months	Pf infections	Placebo	4	27.7	0.14	1	-	
		AQ-SP	1	29.1	0.03	0.24	(0.03, 2.13)	0.3383
		AR-SP	2	29.0	0.07	0.48	(0.09, 2.61)	
	Pv infections	Placebo	4	27.6	0.15	1		
		AQ-SP	3	29.0	0.10	0.71	(0.16, 3.18)	0.2669
		AR-SP	8	28.7	0.28	1.92	(0.58, 6.38)	
After dose 2 at 6 months	Pf infections	Placebo	3	27.3	0.11	1	-	
		AQ-SP	2	29.6	0.07	-	-	
		AR-SP	0	27.4	0.00	-		
	Pv infections	Placebo	27	26.4	1.02	1	-	
		AQ-SP	3	29.7	0.10	0.1	(0.03, 0.32)	< 0.0001
		AR-SP	7	27.2	0.26	0.25	(0.11, 0.57)	
After dose 3 at 9 months	Pf infections	Placebo	9	26.9	0.34	1	-	
		AQ-SP	2	28.4	0.07	0.21	(0.05, 0.97)	0.027
		AR-SP	2	26.8	0.08	0.22	(0.05, 1.03)	
	Pv infections	Placebo	23	26.2	0.88	1	-	
		AQ-SP	5	28.4	0.18	0.2	(0.08, 0.53)	0.0002
		AR-SP	7	26.7	0.26	0.3	(0.13, 0.69)	
After dose 4 at 12 months	Pf infections	Placebo	11	25.6	0.43	1	-	
		AQ-SP	3	28.0	0.11	0.25	(0.07, 0.90)	0.0029
		AR-SP	1	26.4	0.04	0.09	(0.01, 0.68)	
	Pv infections	Placebo	28	24.9	1.13	1	-	< 0.0001
		AQ-SP	5	27.9	0.18	0.16	(0.06, 0.41)	
		AR-SP	13	25.6	0.51	0.45	(0.23, 0.87)	

Table 5-6: Incidence of malaria per treatment arm within 6 and 12 months following the end of the intervention ITT)

		15-21 months					
	Group	**Numbner of cases**	**Time At Risk (PYAR)**	**Incidence per PYAR**	**RR**	**95% Conf. Interval**	**p-value**
All malaria	Placebo	171	149.0	1.15	1	-	
	AQ-SP	164	155.5	1.05	0.92	(0.70, 1.22)	0.7925
	AR-SP	167	157.2	1.06	0.92	(0.70, 1.21)	
Pf infections	Placebo	65	156.2	0.42	1	-	
	AQ-SP	68	162.0	0.42	1.01	(0.69, 1.49)	0.9391
	AR-SP	74	163.8	0.45	1.07	(0.73, 1.56)	
Pv infections	Placebo	115	152.9	0.75	1	-	
	AQ-SP	112	158.8	0.71	0.96	(0.69, 1.33)	0.7715
	AR-SP	106	161.1	0.66	0.89	(0.64, 1.24)	
Anemia (<8g/dl)	Placebo	42	157.7	0.27	1	-	
	AQ-SP	44	163.4	0.27	0.98	(0.62, 1.57)	0.988
	AR-SP	45	165.4	0.27	1.02	(0.64, 1.62)	

Figure 5-3 Prevalence of parastiemia during follow-up visits



Safety

A total of 6,165 adverse events and 275 severe adverse events were observed during the intervention period (3-15mth) with no significant difference between treatment arms (**Table 5.7**). None of them was found to be study related. One child in the placebo group experienced a non-severe (grade 1) skin rash a few days following administration of the second IPTi dose.

In total, thirteen deaths occurred among study participants. One was excluded from the analysis because he/she did not receive any IPTi treatment dose. Nine occurred in Madang and three in Maprik. Out of the twelve remaining, nine occurred during the intervention period. Of these, seven occurred in the placebo, two in the SP-AS and none in the SP-AQ3 group. An additional thee deaths (one in each group) were observed during post-intervention follow-up (p=0.03) The

likely diagnosis as per available clinical and paraclinical data were: one severe *Pv* malaria (density of 12’200/µl) with severe anemia (Hb=5g/dl)and possible lower respiratory tract infection (severe respiratory distress and cough), four lower respiratory tract infections, three dehydrations and malnutrition, one tuberculosis, one meningitis and two with unknown diagnosis.

Adherence and levels of drug resistance

Adherence to study drugs was assessed by field workers visiting the parents at home on every day that an IPTi dose should have been given and reported an adherence rate of more than 90% (data not shown)

Prevalence of molecular markers of drug resistance

The presence of mutations related to SP and AQ drug resistance in *Pv* was assessed on 105 randomly selected asymptomatic infections. (*Pf* data no available at this time)

Of 105 *Pv* infections, 53 were monoclonal based on the genotyping of *pvdhfr, pvdhps* and *pvmdr1*. Five isolates (9.4%) were displaying wild type *pvdhfr* haplotype (F57-S58-T61-S117-I173), 20 (37.7%) a double mutant haplotype (57L-58R-T61-S117-I173), one (1.9%) a triple mutant haplotype (57L-58R-T61-117T-I173) and 27 (50.9%) a quadruple mutant haplotype (57L-58R-61M-117T-I173). All the isolates displayed the same haplotype for *pvdhps*: S382-C383-A553-647P. 36 isolates (67.9%) displayed the *pvmdr1* 976F mutation. Twenty (37.7%) isolates carried the quintuple mutant haplotype *pvdhfr* 57L-58R-61M-117T-I173 / *pvmdr1* 976F associated with AQ-SP resistance.

Table 5-7: Incidence of adverse events, serious adverse events, severe anaemia, hopital admission and deaths for Madang and Maprik at different time points

		3-15 months					
		cases	Time At Risk (PYAR)	Incidence per PYAR	RR	95% Conf. Interval	p-value
All adverse events	Placebo	2035	512.07	3.97	1	-	0.9935
	AQ-SP	2078	519.52	4	1	(0.91, 1.09)	
	AR-SP	2052	518.31	3.96	1	(0.91, 1.08)	
Serious adverse events	Placebo	82	517.2	0.16	1	-	0.8408
	AQ-SP	99	524.68	0.19	1.11	(0.78, 1.56)	
	AR-SP	94	523.47	0.18	1.07	(0.76, 1.52)	
Deaths	Placebo	7	517.407	0.014	1	-	
	AQ-SP	0	524.953	0	-	-	
	AR-SP	2	523.723	0.004	-		

5.5 Discussion

This study in PNG infants provides the first evidence for the efficacy of IPTi with SP-based regimens for the prevention of malaria and anaemia in a non-African population exposed to high levels of non-*falciparum* malaria. IPTi with combination of SP plus 3 days of AQ resulted in a 28% decrease in all malaria episodes and a 25% decrease in episodes of moderate-to-severe anaemia (Hb < 8g/dl). Efficacy increased in children that received at least 3 doses of IPTi during the 1st year of life (38% for any malaria and 41% for anaemia, respectively). In line with local resistance patterns and pharmacokinetic properties SP-AS3 was found to be inferior to SQ-AQ3 (see below for detailed discussion). Overall, the efficacy of IPTi SP-AQ3 in PNG infants is comparable to the 30% efficacy observed in African infants receiving IPTi with SP (Aponte, Schellenberg et al. 2009). Both IPTi treatments were well tolerated and safe. The present data thus provide essential proof-of-principle evidence for the safety & efficacy of IPTi outside Africa.

Prevention of malaria

Overall, IPTi with AS-AQ3 was more efficient in preventing *P. falciparum* than *P. vivax* malaria (ITT 37% vs 22%, ATP 51% vs 31%). This was not unexpected. African IPTi studies demonstrated that the IPTi efficacy is almost exclusively due to the post-treatment prophylactic effect of the drugs given with the preventative effect of IPTi SP almost entirely due to a reduction of malaria in the first 5 weeks after treatment (May, Adjei et al. 2008; Aponte, Schellenberg et al. 2009). Consequently, only IPTi with long- but not short-half live drugs were effective in reducing the risk of malaria in infancy (Gosling, Gesase et al. 2009; Odhiambo, Hamel et al. 2010). In line with the African results, in the present study both SP-containing regimens had similarly high protective effect against *P. falciparum* for the first 35 days (72-82%), resulting in an overall efficacy of IPTi against *P. falciparum* that was towards the higher end of those found in African studies (37% vs 30%, (Aponte, Schellenberg et al. 2009; Gosling, Gesase et al. 2009; Odhiambo, Hamel et al. 2010)).

Although a similar post-treatment prophylactic effects was observed in relation to the risk of *P. vivax* malaria with SP-AQ3 preventing 83% of *P. vivax* episodes in the 1st 35 days following treatment, the overall efficacy of IPTi against *P. vivax* malaria was lower in the SP-AQ3 and absent in SP-AS3 arm. The main reasons for this are the ability of *P. vivax* to relapse from long-lasting liver-stages(Krotoski 1989) and higher levels of SP resistance in *P. vivax*.

P. vivax strains from New Guinea are thought to relapse very frequently (Craige, Alving et al. 1947) and following drug treatment *P. vivax* blood-stage infections are therefore re-established very rapidly. In a concurrently conducted in-vivo drug trial PNG children 0.5-5 yrs treated with SP-chloroquine (3d) and SP-AS3, 65% and 49% respectively had recurrent parasitaemia and 22% and 18% a recurrent clinical episode by day 42 (Karunajeewa, Mueller et al. 2008).Consequently, even if an IPTI is able to both clear pre-existing infections and effectively prevent primary infections during 1st 5 weeks, the first relapse will occur at time when drug levels have dropped below the levels required for prevention of re-infection. Even though PNG children acquire immunity to *P. vivax* very rapidly (Lin, Kiniboro et al. 2010), infants are unlikely to have acquire significant immunity in the 1st year of life and are at risk of clinical disease from both new and relapsing infection during the interdose period.

As SP was never used as a mono-therapy in PNG, there are no estimates of SP in-vivo resistance for either *P. falciparum* of *P. vivax*. However, well-validated molecular makers of SP resistance exist for both parasites. In *P. falciparum* the quintuple *Pfdhfr* 51-59-108 / *Pfdhps* 436-547 is associated with a high degree of drug resistance (Picot, Olliaro et al. 2009) while in *P. vivax* 57L-58R-61M-117T quadruple mutation (irrespective of pvdhps mutations) has been associated with increased risk of treatment failure (Hawkins, Joshi et al. 2007; Marfurt, de Monbrison et al. 2008). The high frequency of the quadruple *pvdhfr* mutation but complete absence of quadruple/quintuple *pfdhfr*/p*fdhps* in infection from iPTI children, therefore indicated that while SP retained a substantial efficacy against *P. falciparum*, its efficacy against *P. vivax* was likely to have been severely compromised.

Although chloroquine resistance *P. vivax* was first described in PNG(Rieckmann, Davis et al. 1989), there is a significantly higher level of 4-aminoquinoline resistance present in *P. falciparum* compared to *P. vivax*. AQ resistant *P. falciparum* was first detected in the early 1980s (Tulloch 1980) and within 10yrs reached >35% RII & RIII resistance (Mueller, Bockarie et al. 2003). The almost complete fixation of the SVMNT pfcrt haplotype and the pfmdr1 86Y mutation that were associated with AQ resistance among the *Pf* isolates(Marfurt, Muller et al. 2008) from the IPTI children demonstrated the continued very high level of AQ resistance. There are only limited data on AQ resistance in *P. vivax* from PNG. In a study in 90', 14% (12/88) of infections (any species) treated with AQ did not show an adequate response, but only one *Pv* out of 18 was RII resistant (Genton, Baea et al. 2005). In 2003/04, although SP-AQ3 was 100% clinically effective, recurrent parasitaemia was observed in 12% of children treated (Marfurt, Mueller et al. 2007) with the *pvdhfr* 57L-58R-61M-117T / *pvmdr1* 976F quintuple mutation associated with treatment failure (Marfurt, de Monbrison et al. 2008). The relatively low level of 976F mutations in *P. vivax* infection observed in IPTi children thus indicated that AQ retains good clinical efficacy against *P. vivax* infection in PNG.

The differential efficacy of IPTi SP-AQ4 and SP-AS3 for the prevention of *P. falciparum* and *P. vivax* malaria are therefore well explained by the above resistance patterns and the pharmacokinetic properties of the different drugs used. Given the high level of AQ resistance and the very short half life of AS (Karunajeewa, Ilett et al. 2004), the efficacy to IPTi for the prevention of *P. falciparum* is therefore likely to be due to the preventative efficacy of SP. The high level of preventative efficacy for 35 days is well in line with the terminal elimination half-life ($t_{1/2\beta}$) of 15.6 and 9.1 days for pyrimethamine and sulphadoxine, respectively in PNG infants(Salman, Kose et al. 2011). With SP unlikely to be efficacious against *P. vivax*, the success of IPTi intervention thus relies on the efficacy of the partner drug. Whereas *P. vivax* parasites are efficiently cleared by SP-AS3, the short half-life of AS provides no post-treatment prophylactic effect and recurrent *P. vivax* infections are very common (Karunajeewa, Mueller et al. 2008). AQ on the other hand has a half-life of ~9

days (Stepniewska, Taylor et al. 2009) and a 3day course should therefore effectively suppress sensitive parasites for 4-5 weeks. The differences in efficacy between IPTi SP-AQ3 and SP-AS3 for prevention of *P. vivax* malaria confirm the observation from African studies that only combinations including efficacious long acting drugs such as SP, AQ or mefloquine are efficient in preventing *P. falciparum* malaria (Gosling, Gesase et al. 2009; Odhiambo, Hamel et al. 2010). In addition, the lack of any protective effect of SP-AS against *P. vivax* but not *P. falciparum* adds further evidence that while SP can retain a considerable effect of low to moderate levels of resistance (Griffin, Cairns et al. 2010), it looses its prophylactic activity at very high levels of resistance (Gosling, Gesase et al. 2009).

Prevention of anemia, severe illness and death

Besides reducing the burden of malaria episodes, IPTi also reduced the risk of moderate-to-severe (Hb < 8 g/dl, 25% in the ITT and 34-41% in the ATP analyses, respectively) and severe anemia (Hb < 5gl). Probably because of the small number of cases, the observed decrease of risk didn't reach statistical significance. These effects are in line with previous findings on the prevention of anemia in Africa(Aponte, Schellenberg et al. 2009) and highlight the important contribution of *P. vivax* infections to anemia related morbidity in highly endemic areas(Genton, D'Acremont et al. 2008).

No difference was observed between the groups in number of SAE's (i.e. all cause admissions plus severe illness). But surprisingly, a significantly lower death rate was observed in the 2 treatment-arms compare to the placebo-arm. Even though this finding is very encouraging, it has to be interpreted with caution. Only 11 deaths occurred during the intervention period with most children dying at home. The cause of death therefore had to be established by verbal autopsy (VA). Based on VA's and available clinical records, a majority of children might have died of severe lower respiratory tract infections. Since Sulfadoxine, being a sulfonamide drug, has a well demonstrated suppressive effects on bacterias (Schürmann and al. 2002), it is thus possible that IPTi with SP might provide some level of protection against invasive bacterial infections such as *streptococcus pneumoniae*.

Safety of SP-AQ3 and SP-AS3

IPTi with both SP-AQ3 and SP-AS3 was very safe. More than 4'000 doses of SP and 6'000 doses of AQ & AS were given and no study drug related AEs were observed. As in earlier studies, no significantly increase in risk of malaria or anemia was recorded after completion of 12 months of IPTi, indicating that IPTi does not impair the acquisition of immunity to both *P. falciparum* and *P. vivax* and lead to a rebound in malaria risk. These data are very reassuring in regards to a possible use of these drug combinations in PNG and add the safety data of pooled analysis IPTi SP safety from African studies(Aponte, Schellenberg et al. 2009).

The use SP as prophylaxis has been criticized because of the potential risk of severe skin reaction of Stevens-Johnson syndroms (White 2005) but by now >5600 children have been treated with SP-containing IPTi regimens in well control clinical trials and not a single drug related severe dermatological event was observed (Aponte, Schellenberg et al. 2009; Gosling, Gesase et al. 2009; Odhiambo, Hamel et al. 2010). On the other hand IPTi with mefloquine while highly effective was not well tolerated with high rates of vomiting (Gosling, Gesase et al. 2009). Given that SP has now been used for several years in pregnant women with good acceptability and tolerability, in the absence of novel drug regimens for IPTi, the combination of SP and AQ is the best, currently available option for IPTi in PNG.

Adherence, acceptability and implementation

The size of the difference in IPTi efficacy between "as to protocol" (ATP) analysis and intention to treat (ITT) population indicates that if the intervention is implemented, it will be important to achieve both high coverage and high adherence to the treatment to achieve a good efficacy.

In both SP-AQ3 and SP-AS3 only the first dose was given as directly observed treatment (DOT). Parents were then counseled on the importance of giving the remaining two doses at home. With this approach a very high rates of compliance (more than 90%) was achieved. As a result, appropriate drug levels

were measured in random sample of study participants on the day after the last IPTi dose (AQ or AS) was administered by parents (Senn *et al*, personal communication). Acceptability by parents and health workers of IPTi was excellent and the administration of IPTi alongside EPI IPTi did not have negative impacts on attitudes to EPI, EPI adherence or existing malaria prevention practices(Pell, Straus et al. 2010).

Finally, cost-effectiveness was also investigated in PNG and has shown encouraging results similarly to what has been found in Africa(Conteh, Sicuri et al. 2010)(PNG data not yet available). Therefore, and in line with what was described in Africa(Armstrong Schellenberg, Shirima et al. 2010), the IPTi intervention seems well suited for implementation in rural and remote settings of PNG and both its acceptability and adherence is likely to be very good.

5.6 Conclusions

This study established the first evidence on the efficacy of IPTi for the prevention of malaria and anemia in a region highly endemic for both *Pf* and *Pv* and provides an essential proof-of-principal that IPTi is an appropriate strategy for the prevention of *Pv* malaria, if an effective, long half-life drug is used. Policy makers should therefore consider this intervention in areas outside Africa where the burden of non-*Pf* infections is important. Given the levels of resistance to SP and AQ in many parts of the Asia-Pacific and America, further studies are needed to investigate other combinations with long acting drugs with a better efficacy in particular against *P. vivax*.

In the PNG context, the combination of SP-AQ3, two long-acting drugs with well match half-lives and good activity against either *P. falciparum* (SP) or *P. vivax* (AQ) is an appropriate drug choice for IPTi and its introduction into the national standard treatment guidelines should be considered. If implemented, it will be important to assure that children receive at least three IPTi doses in the 1st year of life to assure optimal protection against malaria and malarial anemia. The replacement of AQ and SP by arthemeter-lumefantrine as the national 1st line treatment will reduce the selection pressure for resistance against AQ and SP

and it can thus be expected that the (prophylactic) efficacy of two drugs will be retained or as seen in Africa following withdrawal of chloroquine possible even improve (Laufer, Thesing et al. 206). Appropriate monitoring of prophylactic efficacy of AQ, SP as well as possible novels drugs such as DHA-piperaquine should therefore accompany IPTi introduction in PNG.

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6 IMCI supplemented with malaria rapid diagnostic test and intermittent preventive treatment (IPTi): impact on diseases incidence rates and case management in Papua New Guinea.

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Working paper

6.1 Abstract

Background

No studies have looked at the appropriateness of Integrated Management of Childhood Illnesses (IMCI) guidelines in the context of the introduction of malaria rapid diagnostic tests (RDT) and Intermittent Preventive Treatment in infants (IPTi), a so-called IMCI^{+}.

Methods

Making use of the passive case detection of the IPTi trial in Papau New Guinea, the appropriateness of IMCI^{+} on the incidence of disease and case management (diagnostic and treatment) was assessed in PNG young children along an IPTi randomized control trial.

Results

1605 children 3-27 months were enrolled and 8944 illness episodes reported. Incidence rates (episodes/child/year) were: 0.85 for LRTI (95%CI,0.81-0.90), 0.62 for malaria (95%CI,0.58-0.66), 0.72 for GI (95%CI,0.65 -0.93) and 0.08 for otitis (95%CI,0.07-0.09). Introduction of RDTs led to high accuracy of malaria diagnosis compared to expert computer-generated algorithm (□□ 0.99). Clinical diagnosis of LRTI (□□=0.47), gastro-enteritis (□□=0.52) and otitis (□= 0.52) were significantly less accurate ($p < 0.001$). According to IMCI^{+}, 6% didn't received antibiotics when they should have and 19% received antibiotics when they shouldn't have. Re-attendance rates within 14 days following LRTI was 9% when children received antibiotics compare to 8% when they didn't receive ($p=0.44$), rates for gastroenteritis were respectively 8% and 9% ($p=0.51$) . No differences were found in the incidence of non-malarial illnesses between the placebo and IPTI intervention arms (AQ -SP and AS-SP).

Conclusion

IMCI^{+} results in a high accuracy of malaria diagnosis, while the syndromic diagnosis of LRTI is insufficiently accurate to adequately guide treatment. Inappropriate use of antibiotics is common. However, their usage seems not to change the outcome of children with LRTI and GI. Better strategies for the identification of diseases that require antibiotics are needed. Although effective in

preventing malaria, IPTi had no impact on other common causes of morbidity in infancy.

6.2 Background

Worldwide, the leading causes of death in children under five years (excluding peri-natal mortality) are pneumonia, diarrhoea and malaria. These three diseases are responsible for an estimate of 5 millions of deaths yearly with more than 90% of them occurring in Africa and countries with limited resources(Bryce, Boschi-Pinto et al. 2005).At a community level and in outpatient settings, data are scarce, but the few available studies reported that acute respiratory infections, diarrhoea and malaria are similarly responsible for a high burden of morbidity in developing countries, the poorest people suffering the most. (Velema, Alihonou et al. 1991; Roca, Quinto et al. 2006; Deressa, Ali et al. 2007; Animut, Mekonnen et al. 2009; Feikin, Olack et al. 2011)

For decades, a high importance was given to malaria as a cause of fever, which has lead to an overestimation of its burden and at the same time an underestimation of other diseases. (Reyburn, Mbatia et al. 2004; Gwer, Newton et al. 2007) This is still the case in many countries where malaria prevalence is measured through presumptive diagnosis only. Nowadays, case management strategy of sick children under five in developing countries is usually based on clinical presentation of diseases (syndromes) rather than on aetiologies as recommended by the World Health Organization (WHO) who developed the Integrated Management of Childhood Illness (IMCI)(WHO 2007) guidelines for this purpose. For example, cough or difficult breathing associated to tachypnoea defines a lower respiratory tract infection (LRTI), which includes pneumonia of bacterial and viral origins. The estimation of the burden of diseases in developing countries is usually based on data using these syndromic definitions of diseases. In order to get a precise estimate of the burden of syndromic diagnosis in a specific setting, it is crucial to have detailed records of the signs and symptoms of patients attending the clinic at health facilities. Unfortunately, these data are often missing and as a consequence there is only limited information on the incidence of major classes of diseases in young children in tropical countries.

Another well-known problem with the syndromic approach is the overlap of signs and symptoms between different diseases, e.g. malaria and pneumonia.(O'Dempsey, McArdle et al. 1993; Kallander, Nsungwa-Sabiiti et al. 2004). This has important implications for the treatment of these two diseases. It remains unclear how much of this overlap of signs/symptoms is related to children commonly being infected with both malaria parasites plus one respiratory pathogen rather than the overlap in the clinical picture of the individual diseases (O'Dempsey, McArdle et al. 1993; Kallander, Nsungwa-Sabiiti et al. 2004), Gaining a better understanding of the interaction between both diseases in the context of primary health facilities in tropical countries is important for assuring appropriate treatment of both diseases.

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Although the IMCI strategy has been used for many years, few studies investigated the adequacy of its use in routine practice in terms adherence to IMCI recommendations, quality of care and effect on health outcomes. If most experts agree that the introduction of IMCI has improved the quality of care at limited costs(Armstrong Schellenberg, Bryce et al. 2004), IMCI has it own limitations and improving quality of training of health staff is probably not sufficient to achieve a high quality of care (Pariyo, Gouws et al. 2005). Besides the above mentioned problem of overlapping signs and symptoms of major disease classes,, the absence of diagnostic tools, the staff-dependant application of the charts, the availability of resources and the need to take into account the local epidemiology of diseases may all result a in low specificity of IMCI algorithms (Shah and Sachdev 1999; Factor, Schillinger et al. 2001; Armstrong Schellenberg, Bryce et al. 2004; El Arifeen, Blum et al. 2004; Animut, Mekonnen et al. 2009; Horwood, Vermaak et al. 2009; Horwood, Voce et al. 2009).

The advent of sensitive and easy to use, malaria rapid diagnostic tests (RDTs) has the potential to improve the management of febrile patients by providing objective documentation of malaria episodes. Indeed, in regions where microscopy is unavailable, this strategy has the potential to reduce to prescription of antimalarial drugs and improve the treatment of febrile illnesses unrelated to malaria as it was shown in some studies(Skarbinski, Ouma et al. 2009; Ansah, Narh-Bana et al. 2010; d'Acremont, Malila et al. 2010 ; D'Acremont, Kahama-

Maro et al. 2011).With increasing evidence that deciding on antimalarial treatment based on parasitological evidence is safe even in young children, there is strong case for including the use of RDTs into IMCI guidelines.

In addition, novel preventive interventions such as Intermittent Preventive Treatment for malaria in infants (IPTi) have demonstrated to be efficient to reduce the burden of malaria in endemic areas. Given its close link with the Expanded Program of Immunization (EPI), IPTi could be easily implemented through and monitored within the IMCI guidelines.

No studies have evaluated the overall benefits for children' health and health system of integrating diagnostic tools such as RDT for malaria or IPTi interventions to IMCI strategies as an intervention that could be termed as 'IMCI^{+}'.

Similarly to other tropical countries, PNG is suffering a high burden of malaria on costal areas and pneumonia is the leading cause of admission in children under five years in the Highlands.(Shann, Gratten et al. 1984; Duke T, Michael A et al. 2002; WHO 2005) Case management of sick children is almost only syndromic-based using guidelines based on IMCI recommendations. In outpatient settings, few diagnostic tools are available and even microscopy or RDT are rarely available in health facilities to confirm malaria infections. Thus, presumptive treatments with antimalarial and/or antibiotics are prescribed to cover common diseases such as pneumonia, malaria or otitis. In PNG, no data are available on the incidence of diseases and on the performances of the IMCI strategy in outpatient settings.

A randomized controlled trial of Intermittent Preventive Treatment for malaria in infants (IPTi) was carried out from 2006 to 2010 in two regions of Papua New Guinea endemic for malaria. The aim of the trial was to investigate the prophylactic efficacy of a fixed regimen of antimalarial drugs provided four times during the first year of life for the prevention of malaria and anemia (see chapter 5). As part of the trial, a passive case detection system was set up to record illness episodes in all study participants aged 3 to 27 months old. All illness

episodes were managed in accordance with the PNG standard treatments guidelines (local IMCI), except for malaria, where treatment was guided by RDT instead of using presumptive or syndromic diagnosis. This study therefore provides a unique opportunity to investigate the appropriateness of using an IMCI approach to guide case management of common illnesses in young children in the context of introduction of malaria RDT use and IPTi implementation, the so-called IMCI^{+}. In addition, it can also provide important estimates of the burden of common syndromes/diseases as well as information on the potential effect of the IPTi intervention on other diseases than malaria.

6.3 Methods

The study was carried out along the IPTi randomized controlled trial performed in PNG and all data were collected within this cohort of patients.

Study sites and population

The study was carried out in two sites on the north coast of mainland Papua New Guinea (PNG) in Mugil, Madang Province and in the Wosera, East Sepik Province. Each study site included approximately 20 villages (total 40 villages). The 40 study villages are serviced by 4 major health centres: 1) Mugil and Alexishafen in Madang area. 2) Kunjingini and Kaugia in Wosera. Several aid posts were also used for the morbidity surveillance. The enrolment and follow-up were performed from the 6th of June 2006 to the 16th of June 2010; 1605 infants were recruited in the trial. All IPTi preventive treatments were given at time of routine vaccination time points at 3, 6 & 9 months and Vitamin A supplementation at 12 months following the PNG national expanded program of immunization (EPI).

The study was carried out in accordance with Good Clinical Practice (GCP) and monitored by an independent external monitor. The study was approved by the PNG Medical Research Advisory Committee (MRAC number 05.20). The trial was registered on www.clinicaltrials.gov (number NCT00285662) and formed part of the IPTi consortium (www.ipti-malaria.org).Parents or carers were asked to fill a written consent form prior to enrolment of their child in the trial.

Children enrolled in the trial were randomly allocated to receive placebo or two different antimalarial drug regimes [sulfadoxine/pyrimethamine (single dose) with either 3 days of amodiaquine (SP-AQ3) or 3 days of Artesunate (SP-AS3)].The primary objective of the trial was the determination of protective effect of the two IPTi regimens on malaria episodes. Children were recruited into the IPTi trial alongside monthly outreach maternal and child health clinics (MCH). In total, participants were seen actively every 3 months (up to 9 scheduled visits in total) to receive the study drugs or to have blood collected for malaria parasites identification.

Clinical procedure for sick study participants

Throughout the trial, a passive case detection system was maintained at the four study health centres outpatient clinics as well as at four aid posts to assure a continuous morbidity surveillance of study participants. The passive surveillance's records were used to perform the present study. The parents of study participants were encouraged to attend these study clinics free of charge for clinical management whenever their child was sick. Even when children came for scheduled visits (to receive the IPTi intervention), he/she was assessed for sickness only if the mother spontaneously reported that the child was sick.

Each illness episode was assessed by study staff using a standard case report form (See appendix 1). Management of sick children followed the PNG standard treatment guidelines except for malaria and anemia where a specific procedure was adopted to guarantee the best possible care to study participants. Indeed, in case of history of fever in the past 48 hours or an axillary temperature > 37.5°, a rapid diagnostic test for malaria [RDT (ICT Combo®) South Africa] was performed and Hb level measured using a portable Hemocue 201® machine (Angelholm, Sweden). Malaria treatment decision was based on RDT results with only infants with positive results treated with artemether/lumefantrine (Coartem®, Novartis, Switzerland), irrespective of the species. The children with moderate to severe anemia (Hb< 8g/dl) received iron supplementation at the dose of 5mg/Kg of ferrous sulphate for 6 weeks. Pulse oxymetry was also assessed in sick study participants from 2007 onwards. It was recommended to clinical staff that they should consider admitting the child if value drop below 94%. All other illnesses

were treated according to the standard treatment guidelines of PNG, which is based on IMCI (See appendix 2). Children were also assessed for danger signs or symptoms and referred to the health centre for admission when necessary (see appendix 1 for criteria). If children attended after hours, they were taken care by the regular health centre nurses staff. As they did not have the capacity to perform any blood test such as malaria rapid diagnostic test at the time of admission, treatment was delivered on presumptive diagnosis only. On site study nurses recorded their presumptive diagnosis and provided treatment. All records were cross-checked by the study clinicians who confirmed the likely diagnosis. Both study nurses and clinicians could record as many as 3 different diagnoses for each episode. All illness episodes were graded by the study clinicians according to the severity. Detailed trial procedures for malaria & anemia and the main features of the PNG standard treatments are displayed in appendix 2.

Laboratory procedure

A blood sample was collected from finger prick at the same time as malaria and anemia were assessed. These samples were firstly used to perform a blood slide for research read (not used to guide malaria treatment) and secondly samples were stored to undergo in-depth malaria analysis (acquisition of immunity, drug resistance pattern and host/parasites genotyping).

Definition of syndromes and diseases

For the present study, we defined four major syndromes (based on signs and symptoms only) and one disease (documented with diagnostic test) with different levels of severity according to IMCI^{+}:

1. Respiratory infections (syndromes)
    - Acute Respiratory tract Infections (ARI)
        - Upper Respiratory Tract Infection (URTI)
        - Lower Respiratory Tract Infection (LRTI)
            - Severe LRTI
2. Gastro-enteritis (GI, syndrome)
        - Mild GI
        - Moderate to severe GI

3. Malaria (disease confirmed by RDT)
    - Uncomplicated malaria
    - Severe Malaria
4. Otitis

Table 6.1 describes the criteria applied to the different syndromes and diseases with their corresponding treatment as recommended by the national treatment guidelines in detail.

For this study, a definition for malaria based on clinical signs and symptoms plus RDT results was used in order to give estimates of its burden in PNG. In-depth investigations of the safety of using RDT for the diagnosis of malaria and the treatment with artemether/lumefantrine for all species is presented in chapter 4 (Senn et al, in preparation)

Table 6-1: Definition of syndromes or diseases (including the use of RDT for malaria)

Table 1: Definition of syndromes or diseases (including the use of RDT for malaria)

			Criteria	Standard treatment
Respiratory infections				
Acute Respiratory Infection (ARI)			Cough and/or difficulties in breathing	
	Upper Respiratory Tract Infection (URTI)		Cough and/or difficulties in breathing AND RR<50 (<1Y) RR<40 (>1y) AND No respiratory distress (chest indrawing, cyanosis, nasal flaring)	Paracetamol
	All Lower Respiratory Tract Infection (all LRTI)		Cough and/or difficulties in breathing AND RR>50 (<1Y) RR>40 (>1y)	
		mild LRTI	Cough and/or difficulties in breathing AND RR>50 (<1Y) RR>40 (>1y) AND No respiratory distress (chest indrawing, cyanosis, nasal flaring)	Amoxicillin 5 days
		Moderate to Severe LRTI	Cough and/or difficulties in breathing AND RR>50 (<1Y) RR>40 (>1y) AND Respiratory distress (chest indrawing, cyanosis, nasal flaring)	Crystapen im or chloramphenicol im 10 days, admit
Gastro-enteritis (GI)				
All GI			Diarrhoea and/or vomit	
	Mild GI		Diarrhoea and/or vomit AND no dehydration (slow skin pinch, sunken eyes)	more hydration + breast feed
	Moderate to Severe GI		Diarrhoea and/or vomit AND dehydration (slow skin pinch, sunken eyes)	ORS or iv fluid +/- admit
Malaria				
All malaria			Fever and/or history of fever in past 48h AND positive RDT (any species)	
	Uncomplicated malaria		Fever and/or history of fever in past 48h AND positive RDT (any species) AND No danger signs	Artemether/lumefantrine (Coartem) 3 days
	Severe malaria		Fever and/or history of fever in past 48h AND positive RDT (any species) AND At least one danger signs	Artemether im + sulfadoxyne/Pyrimethamine + admit
Otitis				

Study procedure

Illness episodes were included in the study if they fulfilled the following inclusion criteria:

- IPTi study participant
- No illness episode in the past 2 weeks (to avoid counting the same episode twice)

Illness episodes were firstly classified according to syndromic or disease definitions described above in a standardised fashion using IMCI criteria and based on the morbidity records. Episodes not fitting any of the above definitions were categorized as “other diagnosis”. This provided an ‘objective’ measure of the prevalence rates of the morbid episodes in the outpatient clinics.

The severity of the morbid episodes was also assessed by the study clinicians. A serious advers event (SAE) was defined as a illness episode with potential life-threatening features such as respiratory distress or severe pallor. At least one danger sign or symptom need to be present in order to be classified as SAE (as per IMCI, see 10-step check-list, annex 2). A child with criteria for SAE was not necessary admitted.

Health care in Papua New Guinean outpatient settings is based on IMCI. In order to provide appropriate treatment, it is of importance that health staffs apply strict IMCI criteria for diagnosis. Comparing diagnosis as recorded by health workers and standardised post-hoc computerized diagnosis allowed estimating how well the IMCI^{+} version was adhered to. In addition, the prescription of antibiotics was evaluated in detail as a proxy of adherence to the IMCI guidelines by health staff. The impact of antibiotics prescription on health outcomes was investigated by calculating the rate of re-attendance within 14 days and the outcome upon re-attendance (death, serious adverse events or mild diseases) for specific syndromes according to the use of antibiotics. Indeed, as syndromes were not necessary correctly identified by health staff, a certain number of children did not receive antibiotics when they should have and the other way round.

Finally, we were also able to investigate the effect of IPTi on the major syndromes by comparing incidence rates between treatment arms during the intervention period (3-15 months).

Statistical analysis

All data were doubled entered using FoxPro software. Data analyses were performed using STATA software (10.0). In order to describe the spectrum of common syndromes & diseases in an outpatient setting, prevalence rates were calculated based on definitions described above (ARI, URTI, LRTI, malaria, GI and otitis) . More than one diagnosis was possible for each illness episode. Overlap of signs and symptoms of the main syndromes are presented as Venn diagram based on a model developed by Rodgers *et al* (www.cs.kent.ac.uk/people/staff/pjr). Incidence rates and incidence rate ratios were calculated using a negative binomial regression model. In order to avoid counting a single illness episode more than once, children were considered not at risk of the same type of illness category in the two weeks prior to clinic attendance. Following an illness episode, a child was not excluded from time at risk in order to capture a possible interaction between malaria and respiratory infections. Concordance of health workers' diagnosis and that of the standardized definitions was assessed by using kappa test.

6.4 Results

Baseline characteristics

1605 children 3 months old were enrolled and followed-up for up to 24 months between June 2006 and June 2010 in the IPTi randomized controlled trial (Madang=1125 and Maprik=480). 1512 (94%) children presented at least 1 illness episode (Madang=1053 and Maprik=459). The morbidity surveillance reported a total of 8944 illness episodes (Madang=5978 and Maprik=2966). 371 Serious Adverse Events (SAE) and 13 deaths occurred during the study. Overall, 2903 illness episodes occurred in the placebo group, compared to 3038 in the AQ-SP group and 3002 in the AR-SP. **Table 6.2** describes the baseline characteristics of the patients. No significant differences in the demographic characteristics of the children enrolled into the different treatment allocation groups were observed.

Table 6.3 displays the dangers signs/symptoms, clinical and para-clinical features of all illness episodes with full records available. As shown, 81% of the illness episodes presented with fever/history of fever and 77% had cough. No significant difference was observed between the different treatment groups.

Table 6-2: Baseline Characteristics of the study participants

IPTi treatment arm	**total**		**PLACEBO**		**AQ-SP**		**AR-SP**	
N	**1605**		536		536		533	
Mean Age at enrollment (days)	97		96		97		97	
Weight (Kg)	5.8 (5.8 - 5.9)		5.8 (5.7 - 5.8)		5.9 (5.8 - 5.9)		5.9 (5.8 - 5.9)	
Haemoglobin (g/dl)	9.5 (9.4 - 9.5)		9.5 (9.4 - 9.6)		9.5 (9.4 - 9.6)		9.5 (9.4 - 9.6)	
Mean numb. of illness episodes/child (range)	8.4 (0 - 28)		8.1 (0 - 20)		8.7 (0 - 24)		8.3 (0 - 28)	
	%	**(N)**	**%**	**(N)**	**%**	**(N)**	**%**	**(N)**
Girls	47.9	768	51.1	274	44.2	237	48.2	257
Slept under bednet last two weeks *	86.5	1384	87.3	466	85.4	457	86.8	461
Recruitment during rainy season (Sep-Jun)	82.2	1320	83.2	446	81.7	438	81.8	436

* Most of bet nets are untreated (national program started end of 2008)

Table 6-3: Clinical and paraclinical features of all illness episodes per treatment arms with no previous visit in the past 14 days

	all illnesses			Placebo		AQ-SP		AR-SP	
	%	(N)	tot with records	%	(N)	%	(N)	%	(N)
Danger signs									
unable to eat	0.8	62	7980	0.6	16	0.9	25	0.8	21
Vomit everything	0.6	46	7978	0.6	16	0.5	13	0.6	17
Uncsonscious /drowsy	1.5	119	7975	1.4	35	1.6	43	1.5	41
Respiraotry distress	2	159	7971	1.7	44	2.4	66	1.8	49
Fits	1	76	7971	1.1	29	0.9	24	0.9	23
Inability to sit up	0.2	15	7972	0.1	3	0.3	7	0.2	5
Neck stifness	0.1	11	7969	0.1	3	0.11	3	0.2	5
Severe Dehydration	0.7	56	7970	0.5	14	0.7	18	0.9	24
Pale with HR>160 or oedema	0.3	21	7966	0.5	12	0.2	5	0.2	4
Main signs and symptoms									
Fever (temp>37.5° / hist of fev)	81.4	6505	7987	82.2	2140	80.8	2194	81.3	2171
palor	2.6	205	7840	3.1	78	2.2	59	2.6	68
cough	76.8	6110	7954	77.2	2001	77.6	2096	75.6	2013
Difficulties in breathing	15.5	1228	7921	15.6	402	16.1	433	14.8	393
Running nose	46.1	3647	7905	44.7	1153	47.2	1268	46.5	1226
Diarrhoea	21.1	1679	7941	20.7	535	22	595	20.7	549
Abdominal pain	2.8	218	7921	2.8	71	2.7	73	2.8	74
Vomit	10.6	838	7929	11.1	287	10	270	10.6	281
Ear pain	0.7	52	7928	0.6	16	0.6	17	0.7	19
Skin rash	0.3	13	5184	0.2	4	0.2	3	0.3	6
Skin abscess	1	80	7932	1.2	31	1	26	0.9	23
Pus out of the ear	2.5	199	7882	2.4	62	2.7	71	2.5	66
Para-clinical features									
Mean temperature	37.4			37.5		37.4		37.4	
Median heart rate (range)	134	(45-220)		134	(45-208)	134	(54-205)	134	(58-220)
Median resp rate (range)	40	(20-90)		40	(20-90)	40	(20-88)	40	(20-90)
Mean weight	8.6			8.5		8.6		8.6	
mean pulse O2 (range)	98.3	(68-100)		98.4	(73-100)	98.3	(84-100)	98.2	(68-100)
Mean Hb level	9.6			9.5		9.6		9.7	

Prevalence of syndromes and diseases following IMCI+ criteria

A total of 7739 illness episodes had complete records available and 6975 fulfilled the inclusion criteria (no illness episode in the past 2 weeks). Based on standardised definitions of syndromes/diseases (more than one disease per illness episode is possible) 76.5% of episodes (5335/6975, 95%CI 75.5 - 77.5) were diagnosed as ARI, including 44.7% URTI (3116/6975, 95%CI 43.5 - 45.8) , 30.1% mild LRTI (2102/6975, 95%CI 29.0 - 31.2) and 1.7% moderate to severe LRTI (117/6975, 95%CI 1.4 - 2.0). 25.6% had GI (1788/6975, 95%CI, 24.4-29.9), including 24.9% mild GI (1740/6975, 95%CI 23.9 - 26.0) and 0.7% moderate to severe GI (48/6975, 95%CI 0.5 - 0.9). 25.1% had malaria (1750/6975, 95%CI, 23.8- 26.4), including 23.6% uncomplicated malaria (1643/6975, 95%CI 22.6 - 24.6) and 1.5% severe malaria (107/6975, 95%CI 1.2 - 1.8). 2.8% had otitis (199/6975, 95%CI 2.5 - 3.2) and 10.2% (710/6975, 95%CI 9.5 - 10.9) couldn't be classified in one of these syndromes. In this category, there were mainly running nose as single symptom (which cannot be classified as URTI according to PNG guidelines), skin infections and fever without any other accompanying symptom. **Figure 6.1** shows the Venn diagram of the overlap of signs and symptoms among the 4235 illness episodes presenting at least one of the three main syndrome or disease (LRTI, gastro-enteritis and malaria). 32% of the illness episodes fulfilled the definition for more than one syndrome/disease. **Figure 6.2** shows the same Venn diagram for severe diseases only (LRTI, malaria and GI). In this case, only 12% had an overlap of signs and symptoms.

Figure 6-1 Venn diagram showing the overlap of signs and symptoms among 4235 illness episodes with at least one of the three main syndromes

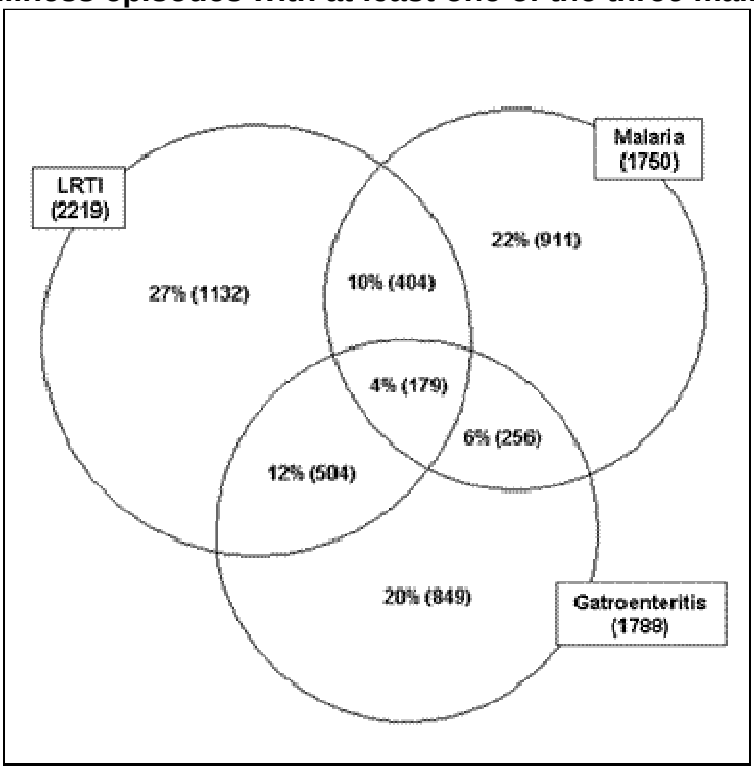
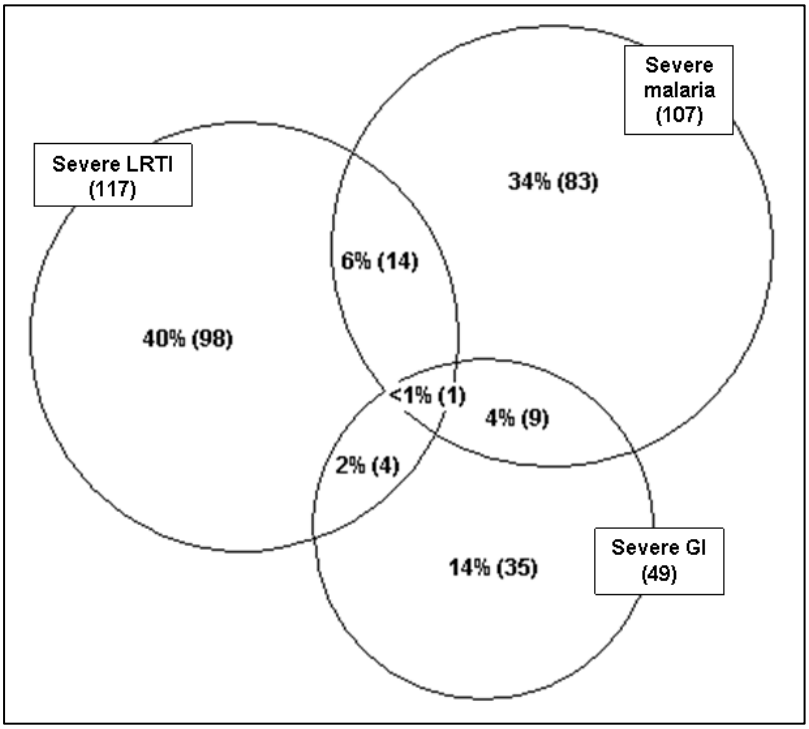


Figure 6-2: Venn diagram showing the overlap of signs and symptoms among 244 illness episodes with at least one of the three main severe syndromes



Correlations (Kappa) between standardised syndromes/diseases and the diagnosis as stated by health staff were 0.42 for URTI, 0.47 for LRTI and 0.52 for both gastro-enteritis and otitis but 0.99 for malaria.

As shown in **table 6.4**, no difference in prevalence rates of different syndromes was observed when events are stratified by season of occurrence (dry season is short from mid July to Mid October)

Table 6-4: prevalence rates for the four major diseases stratified by season (dry season= mid July- until mid-Ocotber)

	Dry			Wet		
	prevalence	CI 95%		prevalence	CI 95%	
LRTI	0.32	0.30	0.34	0.32	0.31	0.33
MALARIA	0.25	0.23	0.27	0.25	0.24	0.26
GI	0.27	0.25	0.3	0.25	0.24	0.26
OTITIS	0.026	0.018	0.034	0.029	0.025	0.034

Incidence rates for the syndromes and disease

With an incidence of 2.05 episodes / child / yr. (95%CI, 1.98-2.12), ARI was the most common syndrome in children 3-27 months old. Of these, URTI accounted for the highest burden with an incidence rate (IR) of 1.2 (95%CI 1.17 - 1.22) followed by LRTI with an IR of 0.85, (95%CI, 0.81 - 0.90). IR of GI was 0.72 (95%CI, 0.65 - 0.93). IR of malaria was 0.62 (95%CI, 0.58 - 0.66) and IR of otitis was 0.08 (95%CI, 0.07 - 0.09).

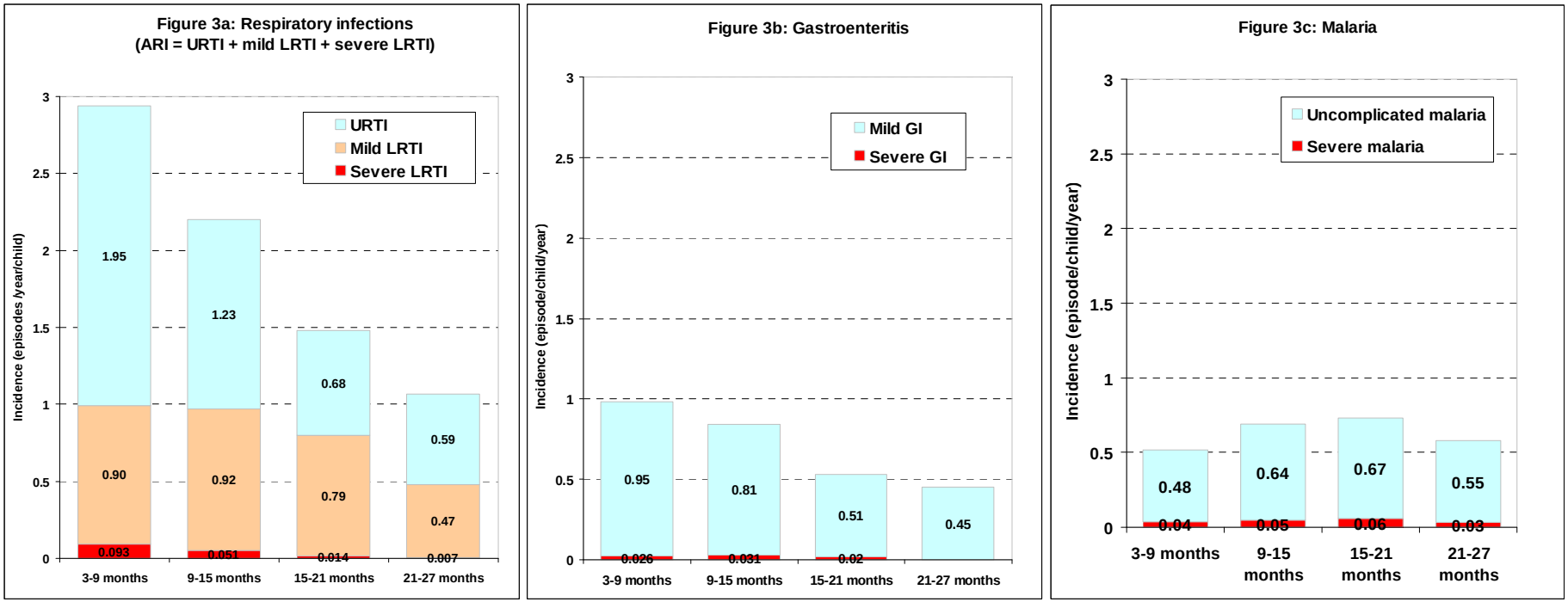
Table 6.5 shows the different incidence rates with confidence intervals for the major non-severe syndromes stratified by age categories in months (3-9, 9-15, 15-21 and 21-27). IR for LRTI and GI are rapidly decreasing with age while the IR for malaria is increasing and no age pattern is observed for otitis (**figure 6.3**).

On average, children experienced 0.13 (95% CI, 0.12 - 0.14) severe episodes/child per year with severe LRTI accounting for a higher burden (IR = 0.05, 95%CI, 0.04- 0.06) than severe malaria (IR = 0.04, 95%CI 0.03 - 0.05) and severe GI (IR = 0.02, 95%CI 0.016 - 0.032).

Table 6-5: incidence rates (episodes/year/child) for the four major syndromic diseases

	3-9 months			9-15 months			15-21 months			21-27 months		
	incidence	CI 95%		incidence	CI 95%		incidence	CI 95%		incidence	CI 95%	
LRTI	0.99	0.95	1.50	0.97	0.92	1.02	0.8	0.76	0.85	0.48	0.44	0.52
MALARIA	0.52	0.49	0.56	0.69	0.65	0.73	0.73	0.69	0.77	0.58	0.54	0.63
GI	0.98	0.94	1.03	0.84	0.8	0.89	0.53	0.49	0.57	0.45	0.41	0.49
OTITIS	0.09	0.07	0.10	0.08	0.06	0.09	0.09	0.07	0.10	0.06	0.05	0.08

Figure 6-3: Incidence rates according to age categories for the main syndromes / diseases: respiratory infections (fig 3a), GI (fig 3b) and malaria (fig 3c)

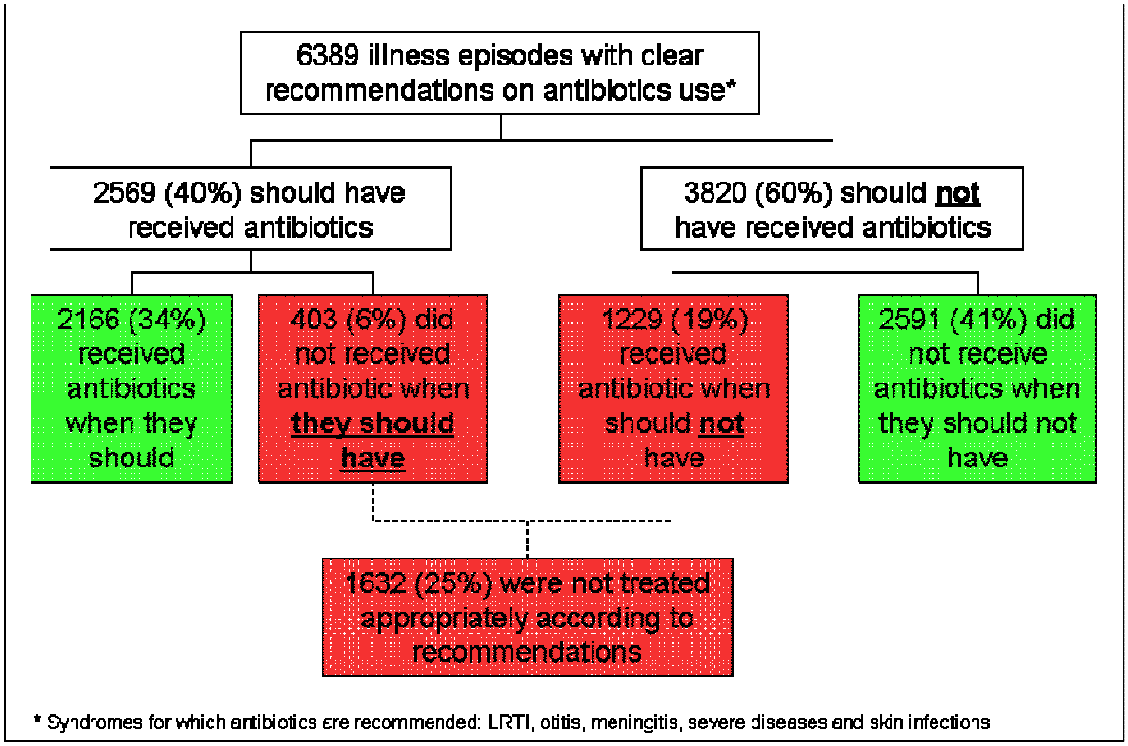


Adherence and safety of IMCI[+]

Out of the 6975 illness episodes fulfilling the inclusion criteria with complete records, 53.6 % were treated by antibiotics (at least one of amoxicillin, co-trimoxazole, i.m. penicillin, i.m. ceftriaxone or erythromycin) and 25.1% received antimalarial drugs (97.6 % for RDT confirmed episodes).

The overall appropriateness of antibiotics was evaluated in 6389 illness episodes for which clear recommendations exist in the PNG national guidelines (**Figure 6.4**). According to these data, 25% of children were not treated appropriately. i.e. 6% did not receive antibiotics when they should have and 19% received antibiotics when they should not have.

Figure 6-4: Appropriateness of antibiotics use according to the IMCI recommendations

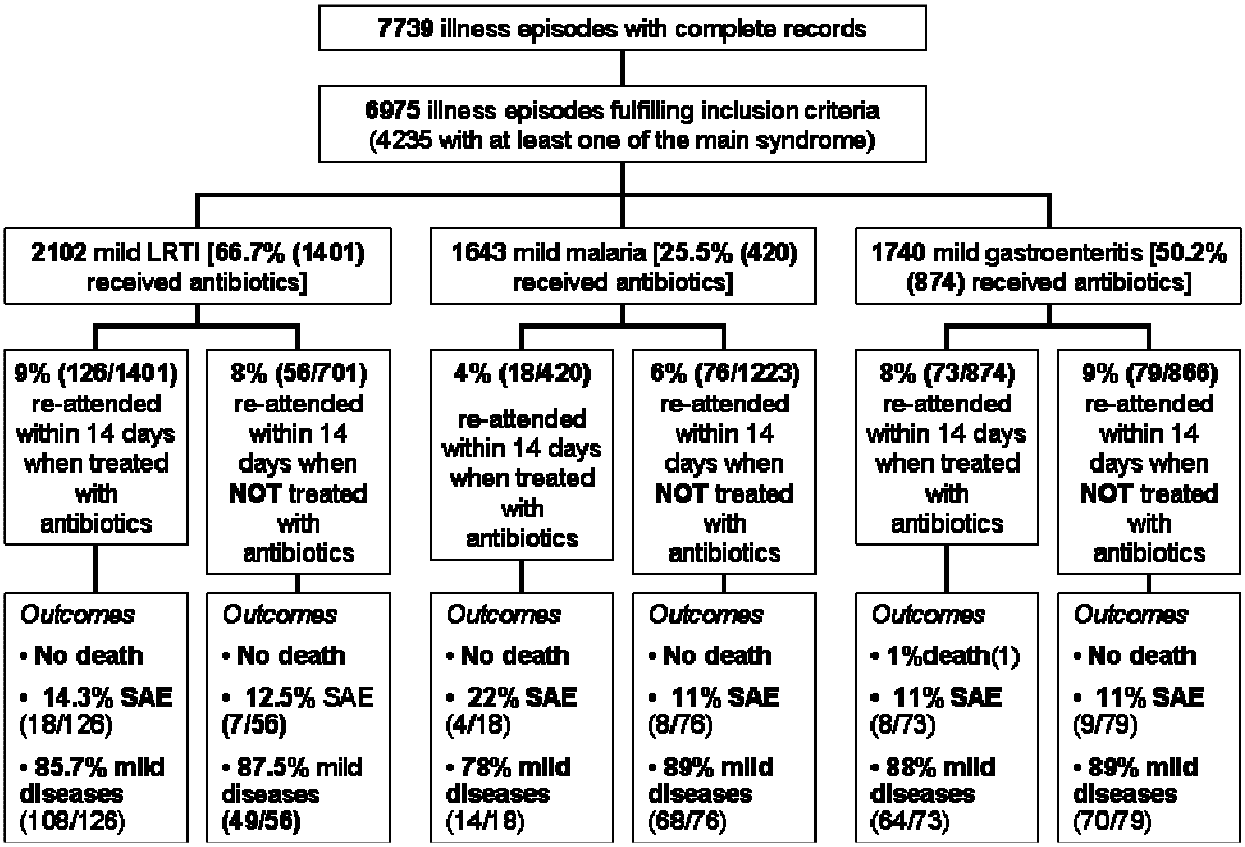


Among children with a clinical diagnosis of URTI 53.4% (1330/2493) received (inappropriately) antibiotics (excluding other reasons for receiving antibiotics) and 66.5% (1351/2031) of mild syndromic LRTI received (appropriately) antibiotics

(excluding severe cases). When children had a diagnosis requiring no use of antibiotics (all except LRTI, otitis, skin infection, meningitis, or gastro-enteritis lasting more than 7 days) the prescription drop from 56% if RDT was negative to 16% when it was positive ($p<0.001$). When children presented with criteria for both LRTI and malaria, only 39.3% of antibiotics were prescribed. In contrast, when they had a single diagnosis of LRTI (without malaria), the prescription of any antibiotics raised to 76.5% ($p<0.001$). Among children with a diagnosis of severe LRTI 96.6% (113/117) received any kind of antibiotics, 75.2% (88/117) received i.m. or i.v. antibiotics as per national guidelines and 49.6 % (58/117) were admitted. 55% (437/795) patients with mild gastro-enteritis received (inappropriately) antibiotics (excluding other reasons to give antibiotics). Finally 90.9% (179/197) children with otitis received (appropriately) antibiotics.

Re-attendance rate within 14 days following a clinical diagnosis of mild LRTI (as per syndromic definition) was 9% when treated with antibiotics compare to 8% when they did not receive any antibiotics ($p=0.44$). For mild malaria, even though not significant ($p=0.14$), a lower number of children came back within 14 days if they received antibiotics, with a re-attendance rate of 4% vs 6% when they did not receive antibiotics. For gastroenteritis, no difference in re-attendance rates was observed ($p=0.51$) whether they received antibiotics or not with 8% vs 9%, respectively. **Figure 6.5** displays the re-attendance rates and outcomes within 14 days following the initial consultation for mild LRTI, uncomplicated malaria and mild GI according to the prescription of antibiotics.

Figure 6-5: Re-attendance rates within 14 days and outcomes for the most common mild syndromes according to the prescription of antibiotics or not



Protective efficacy of IPTi treatments against ARI, all LRTI, severe LRTI & GI infections

The incidence rates of all illness episodes over the entire intervention period (3-15 months) were: 3.37 (95%CI, 3.21 - 3.53) in the placebo group, 3.37 (95%CI, 3.22 - 3.54) in the SP-AQ3 group and 3.31 (95%CI, 3.15 - 3.47) in the SP-AS3 group. Incidence rate ratios (IRR) were similar for both treatment arms with 1.0 (95%CI, 0.92-1.09, p=0.67) for SP-AQ3 and 0.98 (95%CI, 0.90 - 1.07, p=0.99) for SP-AS3

Incidence rates for ARI 3-15 months (intervention period) were: 2.59 (95%CI, 2.46 - 2.74) in the placebo, 2.63 (95%CI, 2.50 - 2.78) in the SP-AQ3 group and

2.54 (95%CI, 2.41 - 2.68) in the SP-AS3 group. IRR were similar with 1.01 (p=0.78) for SP-AQ3 and 0.98 (p=0.63) for SP-AS3.

Incidence rates for all LRTI 3-15 months were very similar in all groups with 0.97 (95%CI, 0.89 - 1.06) in the placebo group, 1.00 (95%CI, 0.92 - 1.09) in the SP-AQ3 group and 0.98 (95%CI, 0.90 - 1.07) in the SP-AR3 group. IRR were 1.03 (p=0.64) and 1.01 (p=0.87) for AQ -SP and AS-SP respectively.

Incidence rates for severe LRTI 3-15 months were 0.09 (95%CI, 0.06 - 0.12) in the placebo group, 0.11 (95%CI, 0.09 - 0.15) in the SP-AQ3 group and 0.12 (95%CI, 0.09 - 0.15) in the SP-AR3 group. The IRR were superior to 1 between the treatment arms and placebo with IRR of 1.34 (p=0.18) and 1.35 (p=0.18) for AQ -SP and AS-SP respectively.

For GI (severe included), the incidence rates 3-15 months were similar in all groups with: 0.94 (95%CI, 0.86 - 1.02) for placebo, 0.92 (95%CI, 0.84 - 1.0) for SP-AQ3 and 0.89 (95%CI, 0.81 - 0.99) for SP-AS3. IRR were not significantly different between treatment arms and placebo with IRR of 0.99 (p=0.85) for SP-AQ3 and 0.95 (p=0.51) for SP-AS3.

The efficacy results of IPTi on malaria are described in detail in chapter 5. The following results were shown: The incidence of clinical malaria between the first dose at 3 months and 15 months was 0.85 per year-at-risk overall and 0.22 and 0.68 for *P. falciparum* and *P. vivax,* respectively. In the intention-to-treat analyses, the protective efficacy against all cases of malaria was 29% ([95%CI, 0.10, 0.43], p = <0.001) in the SP-AQ3 group and 12% ([95%CI, -0.11, 0.30], p = 0.12) in the SP-AS3 group.

6.5 Discussion

Morbidity in PNG infants

This study gives for the first time a comprehensive prospective picture of the morbidity of young infants in PNG in routine practice as measured through IMCI criteria augmented by malarial RDT. With almost 8000 standardized illness records, this study therefore provides an important insight into to the distribution

of common syndromes encountered in PNG. More than 80% of illnesses episodes presented with fever. Respiratory infections were the most common syndromes with 75% of all illness records having criteria for ARI (cough and/or difficulties in breathing) and one third having WHO's criteria for a LRTI (pneumonia or bronchiolitis). One quarter each was diagnosed with malaria and/or gastroenteritis. Acute media otitis was much less frequent with only 3%. Only 10% of the children did attend the clinic without any of these syndromes.

Among the 3 main diseases, the overall incidence rate in children 3-27 months was the highest for LRTI with 0.9 episode /child /year followed by gastroenteritis (0.7) and malaria (0.6).This is somewhat higher compared to findings in African countries. However, direct comparisons are difficult due to different reporting methodology and case definitions (Roca, Quinto et al. 2006; Feikin, Olack et al. 2011). We observed important variation across age groups. Indeed, the incidence of LRTI was decreasing rapidly with age, being twice higher in infants 3-9 months (1.0) compare to children older than 15 months (0.5). A very similar pattern was observed for the incidence of gastroenteritis with a rapid decrease from 1.2 in young infant to 0.4 in children 2 years old. On the other hand, the incidence of malaria was increasing with age from 0.5 in infants 3-9 months to 0.7 in children 15-21 months to decrease again in older children. It is possible that the decrease of malaria incidence observed in older children might be due, at least partly, to the introduction towards the end of trial of ITN in the study area. This effect is however difficult to measure as no records are available on which children receive ITN and which not. Nevertheless, these figures illustrate nicely the different patterns of exposure to specific pathogens and provide and highlight the high susceptibility of the younger infants to respiratory and gastrointestinal infections.

Syndromic approach: the problem of accurate diagnosis

The syndromic approach towards diseases management is widely used and recommended by WHO through the IMCI strategy (and used in the PNG

standard treatment guidelines)(WHO 2000). In the present trial, medical staff were asked to follow IMCI recommendations to decide on treatment except for malaria where treatment was provided only if a RDT was positive for any of the *Plasmodium* species (instead of presumptive treatment). As expected, we observed that the overlap of signs and symptoms is important as one third of illness episodes fulfilled the criteria of at least two of the three main syndromes (LRTI, malaria and gastro-enteritis). The overlap is less important for severe diseases where only 12% of episodes had signs/symptoms for at least two diseases/syndromes. This reflects the lack of specificity of diagnosis based exclusively on signs and symptoms, especially for LRTI and for such diseases. This should theoretically lead to an overuse of drugs. For example, children with fever/cough/positive RDT (malaria) / tachypnoea are classified as having both malaria and pneumonia and thus receive antimalarials and antibiotics.

The diagnosis of LRTI is more complex than that of malaria as no reliable and easy-to-use tests are validated to identify respiratory infections that require the use of antibiotics such as bacterial pneumonia. It has been recently recognized that LRTI are often associated with virus detection and that there is a complex and yet not clear interplay between virus and bacteria in the development of pneumonia (Don, Fasoli et al. 2005; Berkley, Munywoki et al. 2010). Considering the dramatically high burden of respiratory illnesses, especially in very young children, the development of more accurate diagnostic strategies able to identify children with pneumonia requiring antibiotics should be on the top list of priorities (Lim, Steinhoff et al. 2006).The potential benefits of the use of RDT to document viral pathogens such as RSV or *Influenza* need be investigated. Beyond the aetiology of respiratory infections, it would be also valuable to develop tests able to identify children with potential severe (invasive) diseases, which require specific supportive care. In that regard, inflammatory markers such as pro-calcitonin or C-reactive protein (CRP) or other proteins and promising analysis of exhaled gases need to be considered(Schmidt, Bhandari et al. 2010).

We observed only a moderate agreement (kappa = 0.47) for the diagnosis of LRTI between the clinical judgment of health workers and a standardize post-hoc

diagnosis (applying signs of symptoms as recorded on forms). This discordance might be partly due to the use of stethoscopes along the study by nurses not familiar with this tool which may have led to an overestimation of abnormal sounds and hence clinical diagnosis of LRTI. Similarly, when standardized criteria were applied for the clinical diagnosis of gastroenteritis and otitis, the agreement with the clinical judgement of health workers remained limited. In contrast, the agreement for malaria was almost perfect (kappa=0.99) which is certainly due to the systematic use of RDT that provides objective results. It is also true that the present work was carried out alongside a malaria drug trial and strong emphasis was put on this disease and its diagnosis & management.

Finally, the syndromic approach for clinical diagnosis is clearly insufficient for some common diseases. For example, dengue fever, with its potential severe outcome, is responsible for almost 10% of fever episodes in coastal PNG in a recent study(Senn, Luang-Suarkia et al. 2011) but is not considered by IMCI guidelines. Similarly urinary tract infections are only seldom identified by this mean.

Adherence to IMCI^{+}

One of the aims of this assessment was to study how IMCI translates into routine practice if used in conjunction with RDT and IPTi. We investigated two aspects: 1) how accurate is the clinical judgment of health workers (see above) and 2) are drugs (mainly antibiotics) appropriately prescribed? The prescription of antibiotics can be considered as a proxy for guidelines' adherence. We observed a high rate of prescription in our cohort as more than half of cases were treated with antibiotics when only a maximum of 40% should have received antibiotics according to guidelines. This is high compared to other similar settings where it was estimated that approximately 20% of patient should receive antibiotics when diagnosis was made by highly skilled clinicians based on IMCI criteria.(Horwood, Voce et al. 2009; D'Acremont, Kahama-Maro et al. 2011) This might reflect the relative inaccuracy recognition of signs & symptoms by health staff. For example, it is likely that tachypnoae is not often properly identified due to wrong respiratory

rate estimation. We observed indeed, that despite the official recommendation of counting over 1 minute and most of respiratory rate were even numbers (Senn & Rarau pers. Comm.) suggesting that health workers are not counting over one minute. Similarly it was sometimes observed that one single respiratory cycle (inspiration and expiration) was counted as two. All these observation might contribute to overestimate fast breathing and consequently the prescription of antibiotics. This is especially a concern as tachypnoea is corner stone of LRTI identification in IMCI.

Overall, we observed that 1 patient out of 4 was not treated according to the recommendations (received antibiotics when he/she should not have and vice versa, see **figure 6.4**). When looking at prescriptions of antibiotics for respiratory infections, it is surprising to observe that half of children with criteria only for URTI (excluding other potential reasons for receiving antibiotics) still received anti-bacterial chemotherapies. On the other hand, we observed that only two third of children with objective criteria for a mild LRTI received antibiotics. This might be explained by the strong emphasis put in the study on malaria. Indeed, when the diagnosis of malaria was made, most of the children received only antimalarial drugs even if they also met criteria for a mild LRTI (we already mentioned that the overlap of signs and symptoms for both diseases was important, around 15%). One might argue that such a high rate of non-adherence to standard IMCI recommendations might also leave a lot children with potential bacterial pneumonia untreated and hence poor outcome. However, we observed that it is in fact not the case. Indeed, first the rate a re-attendance following a visit for mild LRTI was similar irrespective of the use of antibiotics (9% if they received antibiotics and 8% if they did not). This is well in line with findings in Pakistan where the use of amoxicillin was not found to have any benefits on pneumonia as defined by WHO(Hazir, Nisar et al. 2011). Second when the children looked more severely ill with danger signs such as chest indrawing, almost all children were treated with antibiotics (mostly intra-muscular). The absence of difference in the re-attendance rate is probably due to the fact that the vast majority of mild LRTI had a viral aetiology rather than bacterial and the lack of specificity of the

present criteria to identify true pneumonia. In a sub-sample of these children, RDT made on nasal swabs revealed that more than half of children with criteria for LRTI were positive for at least one of the four most common respiratory viruses (Influenza A&B, *Adenovirus* and Respiratory Syncytial Virus). Similar findings have also been published in other countries.(Berkley, Munywoki et al. 2010). Being a malaria drug trial, the present study was not suitable for investigating the prescription habits of health workers for malaria as specific guidelines were used (only patients with positive RDT results were treated using artemether/lumefantrine). However, this study provides still good evidence that the introduction of RDT associated to adequate training of health staff led to a reduction of antibiotics prescription. Indeed, sick study participants with signs/symptoms of LRTI and a positive RDT had significantly less antibiotics prescribed compared to those with a positive RDT (76% vs 39%).

Impact of IPTi on morbidity

Even if the study was not aimed at assessing the efficacy of IPTi on the overall morbidity and for ARI, we could estimate its impact on these outcomes. The efficacy of IPTi on malaria is well documented in Africa (Aponte, Schellenberg et al. 2009) and similar efficacy results were also found in PNG within the present cohort of patients with an overall reduction of the risk of malaria of about 30% (Senn et al, submitted for publication). However, out of the same cohort of patients, we do not observe any reduction of the overall morbidity following the IPTi intervention. Indeed, the incidence of illness episodes was similar in all trial arms as well as the mean number of illness episodes. More specifically, no protective efficacy was observed on mild LRTI in infants 3-15 months (duration of the IPTi intervention) as incidence rates were similar. There was some weak evidence for severe LRTI to be more frequent in the two treatment arms compared to the placebo group. Again, the study was not designed to assess the efficacy of IPTi on the morbidity; however, it seems important to take into account this apparent lack of impact of the IPTi intervention on the overall morbidity to assess the true benefit of this intervention.

These data are important to consider from a public health perspective. If it is clear that IPTi has a proven effect on malaria (see chapter 5); however, the fact that no benefit is observed on the overall morbidity and on severe illnesses/hospitalisations is a strong limitation to its implementation nationwide. Indeed, what would be the interest for health authorities to invest resources in an intervention that is neither going to improve the overall health status of children nor going to decrease the workload of health workers because the children are going to be as often sick as before?

Limitations of the study

This study has some limitations. Firstly, being a drug trial, the clinical management of malaria episodes was performed in ideal conditions (i.e. treatment of only RDT positive cases). However, for all other diseases, the recommendation made to the study nurses was identical to the national standard treatment protocols. Secondly, the increased training and monitoring of all clinical activities that was done as part of the trial may have led to an improvement in the overall quality of care. It was indeed recently shown that intensive training in conjunction with monitoring of adherence to guidelines has the potential to improve quality of care, at least in rural hospital.(Ayieko, Ntoburi et al. 2011) It seems therefore essential to put a strong emphasis on improving the training of routine staff with regular updates, especially if RDTs' should be implemented in the PNG standard treatment guidelines.

Third, because this study was carried out alongside a drug trial, it is possible that parents of sick study participants were more likely to attend the clinic. This may have resulted in a higher incidence rates of LRTI compared to other regions, such as Kenya where an incidence of 0.3-0.5 episode/child/year was reported in children under five(Feikin, Olack et al. 2011) (compared to 0.9 in our cohort). However, being a passive case detection, only truly sick study participants were seen at the clinic. Finally, it is possible that the fact that the diagnostic of malaria was based on RDT makes the comparison of incidence rates with other syndromes (LRTI, otitis, GI) more difficult. Indeed, as syndromes were

exclusively based on signs and symptoms, it is possible their incidence rates are slightly overestimated compared to the one of malaria.

6.6 Conclusions

The present study provides for the first time a comprehensive picture of the burden of common diseases in Papua New Guinean infants. The most common diseases in very young infants are respiratory infections followed by gastroenteritis and malaria. Whereas malaria is more common in older children (>15 months), LRTI and gastroenteritis are rapidly decreasing after one year of age. Despite the fact that mild clinical LRTI are often missed by health workers, the prescription of antibiotics in children with syndromic LRTI does not seem to change their outcomes in terms of re-attendance and complications compared to those who did not receive antibiotics. This absence of benefits of antibiotics is certainly due the lack of specificity of the present recommendations to discriminatively differentiate children with true bacterial LRTI and those with viral infections. It is urgently needed to develop better strategies to improve the identification of LRTI that require antibiotics or more intensive clinical care.

The use of RDT for malaria seems to have improved significantly the quality of diagnosis and is easily implementable within IMCI strategy. On the other hand and surprisingly, the present study demonstrated that the IPTi intervention neither had an impact on the overall morbidity nor on respiratory infections. Policy makers should consider this aspect of IPTi if planning a possible implementation of the intervention in PNG

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7 Community response to intermittent preventive treatment of malaria in infants (IPTi) in Papua New Guinea

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7.1 Abstract

Background

Building on previous acceptability research undertaken in sub-Saharan Africa this article aims to investigate the acceptability of intermittent preventive treatment of malaria in infants (IPTi) in Papua New Guinea (PNG).

Methods

A questionnaire was administered to mothers whose infants participated in the randomised placebo controlled trial of IPTi. Mothers whose infants participated and who refused to participate in the trial, health workers, community reporters and opinion leaders were interviewed. Men and women from the local community also participated in focus group discussions.

Results

Respondents viewed IPTi as acceptable in light of wider concern for infant health and the advantages of trial participation. Mothers reported complying with at-home administration of IPTi due to perceived benefits of IPTi and pressure from health workers. In spite of patchy knowledge, respondents also demonstrated a demand for infant vaccinations and considered non-vaccination to be neglect. There is little evidence that IPTi has negative impacts on attitudes to EPI, EPI adherence or existing malaria prevention practices.

Conclusion

The degree of similarity between findings from the acceptability studies undertaken in sub-Saharan Africa and PNG allows some generalization relating to the implementation of IPTi outside of Africa: IPTi fits well with local health cultures, appears to be accepted easily and has little impact on attitudes towards EPI or malaria prevention. The study adds to the evidence indicating that IPTi could be rolled out in a range of social and cultural contexts.

7.2 Background

Intermittent preventive treatment (IPT) of malaria involves the administration of treatment doses of an anti-malarial drug at predetermined intervals, regardless of parasitaemia or symptoms. IPT during pregnancy (IPTp) is linked to ongoing routine antenatal care and IPT for infants (IPTi) is delivered through the Expanded Programme of Immunization (EPI) (Egan, Crawley et al. 2005).

Various studies in sub-Saharan Africa have shown that IPTi with sulphadoxine-pyrimethamine (SP) given at the time of routine vaccinations in the first year of life reduces the incidence of clinical malaria by between 20% and 59% (Schellenberg, Menendez et al. 2001; Chandramohan, Owusu-Agyei et al. 2005; Pool, Munguambe et al. 2006; Grobusch, Lell et al. 2007; Kobbe, Kreuzberg et al. 2007; Mockenhaupt, Reither et al. 2007), and by 30% across the six sites (Aponte, Schellenberg et al. 2009). In addition to the clinical effectiveness of IPTi, recent social science research has also demonstrated that in several sites across sub-Saharan Africa, IPTi is socially and culturally acceptable (Pool, Munguambe et al. 2006; Pool, Mushi et al. 2008; Gysels, Pell et al. 2009). This acceptability research, undertaken alongside clinical trials (in Gabon, Kenya, Mozambique and Tanzania) and implementation studies of IPTi (in Ghana, Malawi and Tanzania) with various drug regimens, also indicated that IPTi does not negatively influence attitudes to and uptake of immunization, nor is IPTi misunderstood as immunization against malaria. IPTi does not, therefore, influence other preventive measures or delay treatment seeking for malaria.

Although the acceptability research published to date provides a comprehensive insight into community responses to IPTi delivered in sub-Saharan Africa, there are no published data on the acceptability of IPTi outside of sub-Saharan Africa. Given that IPTi has the potential to reduce the malaria-related morbidity and mortality for infants in other regions with a significant level of malaria mixed

endemicity (*Plasmodium falciparum* and *Plasmodium vivax*), such as South-East Asia or Oceania, it is crucial to ensure that IPTi is socially and culturally acceptable in distinct social and cultural settings outside of sub-Saharan Africa.

Building on previous acceptability research undertaken in sub-Saharan Africa and in the context of an IPTi trial in Papua New Guinea (PNG), this article aims to:

1. Describe knowledge, perceptions, experiences and responses relating to IPTi and EPI of trial participants, community members, and local health care providers
2. Identify and understand the mutual interactions between perceptions of, attitudes to and experiences with EPI and IPTi.
3. Identify and understand local barriers to the acceptance of and long-term adherence to IPTi.
4. Identify wider socio-cultural, national and regional factors that affect, or may affect, the implementation or acceptability of IPTi.

Setting

The acceptability research undertaken in PNG was carried out alongside a randomized placebo-controlled clinical trial of IPTi, which was run by the Papua New Guinea Institute of Medical Research (IMR) and carried out under the auspices of the IPTi Consortium with the financial support of the Bill and Melinda Gates Foundation. The trial tested the following drug regimens: single dose of SP associated to three days of either amodiaquine or artesunate given four times at three, six, nine and 12 months following the PNG EPI schedule. Each dose of the three-dose regimen was given on consecutive days: trial staff gave the first dose; the second and third doses were given to mothers to administer to their infants the following days. The study drugs were administered in collaboration with local health facilities responsible for operating Mother and Child Health (MCH) clinics, which, due to the scattered nature of settlements in PNG, deliver EPI through monthly village outreach services.

Although the trial commenced in two areas of PNG, East Sepik Province and Madang Province, in 2008, the East Sepik site was closed due to a lower than excepted malaria incidence. The acceptability research therefore focused upon Madang Province, a mainly coastal lowland (0-500m) with a high year-round malaria transmission rate. The study was carried out about 60km north of Madang town.

PNG is renowned for its linguistic and cultural diversity, and in the study area six languages coexist. However, this contrasts with the lack of socio-economic differentiation in rural areas. In Madang province, subsistence farming of a range of crops (including sweet potatoes, greens and tropical fruits) is the main livelihood activity. In addition, both men and women migrate to Mandang town, which is one of the few urban centres in PNG.

7.3 Methods

The methods employed in this study were based on those used in the acceptability studies carried out in sub-Saharan Africa (Pool, Munguambe et al. 2006; Pool, Mushi et al. 2008; Gysels, Pell et al. 2009). In PNG, emphasis was placed on a questionnaire survey of mothers whose infants participated in the IPTi trial. Additional individual in-depth interviews and focus group discussions with mothers, health workers, village reporters (community members employed by the IPTi trial recording study drug adherence and the occurrence of adverse events), opinion leaders and community members were also undertaken in order to triangulate findings from the questionnaires. The topics explored in the data collection tools were drawn from previous studies and modified to suit the context. These topics included, amongst others: knowledge, understanding and attitudes regarding vaccination; vaccination behaviour; experiences of vaccination; and, knowledge and understanding of IPTi. The behaviour of health

workers and participant mothers was also observed during delivery of IPTi and field notes taken.

Respondents were drawn from four villages within the IPTi trial recruitment area. Initial sampling was based on convenience, e.g. mothers who were willing to be interviewed were recruited through study clinics. As the study developed theoretical sampling was used: participants were recruited on the basis of the research team's developing understanding of the field and the questions that emerged from the ongoing analysis of already collected data. The numbers of questionnaires, interviews and FGDs (see **Table 7.1**), were determined by the point of saturation (when no more novel information emerged). Prior to their participation, informed consent was obtained from all study respondents in accordance with local ethics committee regulations**.** Interviews were carried in Tok Pisin (formerly, Pidgin English) or English, and with the agreement of respondents, recorded and transcribed verbatim, and, when necessary, translated into English by field workers for analysis.

Table 7-1: Study respondents and data collection tools.

Type of respondent	Data collection tool*	N
Participant mothers	IDI	23
	QNN	213
Mother refusers	IDI	2
Health workers	IDI	5
Village reporters	IDI	2
Opinion leaders	IDI	2
Community members	FGD	6

*IDI: in-depth interview; QNN: questionnaire; FGD: focus group discussion

Qualitative data analysis was carried out employing a Grounded Theory approach (Glaser and Strauss 1967), with more abstract generalizations emerging from the data based on the coding of emerging themes using QSR

Nvivo 2. Descriptive statistics from the qualitative data were generated using SPSS 14.0.

Overall ethics clearance for the acceptability research was obtained from the Ethics Committee of the Comité Ético de Investigación Clínica of the University of Barcelona. Separate local ethical clearance was obtained from the PNG Medical Research Advisory Committee and the PNG Institute of Medical Research Institutional Review Board (IRB), approval number 0807.

7.4 Results

Acceptability and adherence

A number of key factors have emerged from the data that influence the acceptability of IPTi in positive or negative ways. These factors relate to local attitudes towards infant care and vaccinations, the clinic environment, the IPTi regimen and the nature of the clinical trial context.

Local health culture and the routinization of EPI

The interviews with mothers whose infants participated in the study indicate that there is common awareness of and demand for EPI vaccinations. Although the mothers were unable to name all the diseases that vaccination prevents (56% [119/213] could name some), they knew that vaccination prevented diseases or provided protection (91% [194/213]), were positive towards vaccination and often distressed by the possibility that their children may miss out on vaccination. Although over 20% (47/213) of mothers stated that vaccination provides prevention against malaria, it is unclear to what extent this was due to confusion with the IPTi study. Vaccinated children were viewed as healthy; indeed keeping the child healthy was cited as the reason for vaccinating children by over 40% of mothers (87/213). There was debate about whether vaccination provided complete or partial prevention for diseases, with general opinion leaning towards partial prevention: vaccination gave some protection, but complete prevention

depends on other factors; the disease would develop more slowly giving mothers chance to go to the clinic; or, the child could be sick again in the future.

Reports of vaccination-related side effects were minimal (2% [5/213] of participant mothers reported side effects), and descriptions focused on mild fever or passing distress. Although relatives and neighbours were said to complain occasionally of children crying after vaccination, mothers were able to dismiss these complains and they did not prevent them from vaccinating their children. Children receiving multiple injections during a single clinic visit was said to cause concern amongst mothers and relatives. Sometimes relatives questioned mothers about the number of injections that the child had received and said that two was too many. One mother reported getting angry after seeing blood drip down her child's arm after vaccination.

In spite of the concerns about multiple injections, very few mothers reported factors preventing them from vaccinating their children (one reported that if she was too busy she may be unable to take her children, another that her husband could prevent her, one mentioned transport problems and one described how she would not be able to go if she was sick). However, in the qualitative interviews, other factors mentioned included: health workers reprimanding mothers for missing previous appointments; difficulties in meeting the costs levied at the clinic; and, laziness.

Although the data suggest that mothers are responsible for the healthcare of the children, taking them to the clinic and caring for them in the home, fathers often play the role of ultimate decision-maker, to whom women have to seek permission to travel to the clinic. Fathers also take a more prominent role in emergencies. Whilst respondents did not report that husbands prevented them from vaccinating their children, there were scattered reports, offered by mothers, of other women's husbands preventing their wives from participating in the IPTi trial. However, one health worker suggested that this might be an easy excuse to

avoid refusing directly. The women who participated in the trial reported having made the decision to participate with their husbands, who agreed because they saw the benefits.

The data revealed only scattered accounts of the use of non-biomedical means of preventing disease: two mothers reported using “paw paw” seeds to prevent disease, and one mother specifically mentioned preventing malaria; one mother referred to herbs more generally; and, a health worker described the use of amulets for spiritual protection by members of the local Catholic church. Herbal remedies were more popular for curing rather than preventing: respondents described a range of remedies for mild diseases such as cough, cold, diarrhoea and fever. Mothers, however, preferred that serious diseases be dealt with in the clinic.

IPTi drugs, adherence and reasons for refusal

Two-thirds of mothers interviewed knew that the IPTi trial involved malaria prevention (67% [143/213]) and almost three-quarters knew they were participating in research (74% [157/213]). However, the vast majority (87% [186/213]) were unsure about the names of the drugs used, and only four named Fansidar (the brand name for SP) as one of the study drugs. Nevertheless, the mothers were almost universally content with their experiences of the trial: the IPTi drugs, the outreach clinics and the free treatment. Mothers reported a decrease in malaria amongst the participating infants and commented more generally on the health of the infants, comparing it favourably with that of other children who did not participate. They also reported that study participation had brought broader benefits as they travelled less often to the clinic and had more time to do other activities such as work. Indeed, mothers commonly enrolled more that one of their children in the study. Trial staff were considered diligent and caring, compared to other health staff who were rude, slow to deal with mothers, and did not give proper care. One mother conceded that this might be due to the health workers’ heavy workload. A common concern expressed by

respondents was what would happen when the IPTi trial came to an end in the area.

Reports of side effects from the IPTi drugs were also rare: two mothers referred to the child's body being weak, but that they quickly recovered; a third, described how her child was seen by one of the trial doctors after suffering a reaction to the trial drugs. In none of the cases was the mother discouraged from continuing her participation.

IPTi was delivered in three doses at four intervals to infants who participated. Two doses were left with the mother to give to the child the following days. Fifteen percent of mothers (31/213) reported that they sometimes did not give the IPTi drug as instructed. The majority, who reported having followed the instructions, did so out of the belief that the drugs would help the child and not doing so would be akin to neglect or due to pressure from health workers.

Respondents reported that during the trial and particularly the recruitment phase there were reports that blood sampled from participants was being sold by the local research institute. People with less experience of research, such as men, the elderly and the residents of remote villages, were said to spread these rumours. Indeed, the majority of participant mothers surveyed reported that the blood was used for diagnostic purposes (62% [133/213] referred specifically to testing for malaria). One woman, who refused to participate in the IPTi trial, did so because she believed that the blood would be used for transfusion, something that she disagreed with. Although there were concerns from two participants about their children losing blood and 16% (33/213) of mothers were unsure about the purpose of the blood sampling, many mothers paid little attention to these complains. The majority of the mothers who refused to participate or dropped out from the study did so because of the rumours associated with blood sampling and not because of IPTi per se.

The influence of IPTi on disease prevention

There is no evidence that IPTi had a negative impact upon EPI adherence. Given the support that the staff employed by the clinical trial gave to the local healthcare system, in the local clinics and at monthly outreach visits to the communities, it is likely that EPI adherence increased during the trial. Furthermore, all participant mothers were positive about their participation.

Participant mothers perceived IPTi as offering complete prevention from malaria: 98% (208/213) of mothers stated that after IPTi their children would not get malaria. This finding is however contradicted by the extent of bed net usage: over three-quarters (77% [163/213]) of participant mothers reported using bed nets as a means of malaria prevention. There was however debate about the effectiveness of bed nets. The new bed nets that were being handed out to trial participants were considered effective at repelling the mosquitoes, in contrast to the less useful older nets. One health worker attributed the popularity of bed nets, to previous campaigns of mosquito eradication that had left a strong message about the importance of preventing mosquito bites.

7.5 Discussion

In a similar fashion to acceptability studies undertaken in Africa (Gysels, Pell et al. 2009), the data from PNG indicate a general concern about infant health and that mothers were generally motivated to comply with prevention practices, regardless of whether they fully understood them or not. Although mothers who participated in the IPTi trial were often not entirely clear about the diseases that vaccinations prevented, they viewed EPI non-compliance as neglect. This was based on the general distinction that they drew between the health of un-vaccinated and vaccinated children. Although references to traditional prevention practices were very rare in the PNG data (a finding which differentiates PNG from other sub-Saharan African sites (Pool, Munguambe et al. 2006; Pool, Mushi et al. 2008; Gysels, Pell et al. 2009)) the observed benefits of vaccination,

together with the general concern about infant health, formed a favourable context for the acceptance and routinization of both EPI and IPTi.

The presence of the IPTi trial in PNG did not negatively affect participants' attitudes towards EPI vaccinations, nor did it influence their other malaria prevention practices, as was the case in the sub-Saharan African sites (Pool, Munguambe et al. 2006; Pool, Mushi et al. 2008; Gysels, Pell et al. 2009). However, whilst in the sub-Saharan African sites, the preventive healthcare practices, including IPTi, were almost always considered as bestowing only partial protection, in PNG mothers viewed IPTi as providing complete protection from malaria. Although one may expect a decrease in bed net usage to accompany this finding, no such reduction was reported. This may be the result of the general compliance with preventive practices or to avoid nuisance mosquito bites. However, if IPTi were implemented across PNG and delivered as part of EPI further research on bed net usage would be useful to confirm this finding.

One of the main determinants of acceptability is whether recipients and other community members perceive the intervention as performing as they think it is supposed to, and whether the benefits are perceived to outweigh the disadvantages. In the case of the clinical trial of IPTi in PNG, participant mothers understood that IPTi was intended to prevent malaria in infants and they identified a positive effect upon the health of participating infants and perceived a decrease in malaria incidence. In a similar fashion to the clinical trial sites, where acceptability studies have been undertaken (Kenya, north-eastern Tanzania, Mozambique and Gabon (Pool, Munguambe et al. 2006; Pool, Mushi et al. 2008; Gysels, Pell et al. 2009)) participant mothers and other community members viewed the benefits of trial participation, which included the free high-quality health care provided by the trial staff at local clinics and during outreach services, and the perceived reduction in malaria amongst participating infants as

outweighing any concerns about the blood sampling and associated rumours of “blood selling”.

Because, in clinical trial settings, it is difficult to isolate the advantages and disadvantages linked to trial from those linked to the intervention, it is challenging to distinguish what precisely motivates mothers to attend clinic and adhere to treatment, and this makes it difficult to assess how they would respond in a more routine implementation setting. However, given the similarities with the response in the intervention sites in Africa it seems likely that the response in “natural” settings will not be very different. In the intervention studies with SP in Malawi and Ghana, ‘IPTi was simply an unnoticed addition to an already routinized EPI’ (Gysels, Pell et al. 2009).

During the PNG trial of IPTi participants had greater opportunity for non-adherence than in the other clinical trials of IPTi: mothers were given the two last doses of the trial drug to administer themselves at home. Although perhaps inflated by desirability bias, the majority of mothers reported having always given the doses as instructed, motivated by the benefits they perceived that the previous doses had proportioned their child (or children). Although a single dose drug regimen for IPTi would be ideal, in order to avoid potential non-adherence, the results from this study suggest that if they perceive benefits, mothers are prepared to administer drugs for prevention unsupervised. Data from other acceptability studies, where health workers sometimes provided IPTi drugs for mothers to administer at home, despite being instructed not to, suggest that some mothers did give the drugs correctly. Other mothers forgot and some, given that they thought the drug was to cool the post-vaccination fever, only gave if the child had fever (Pool, Mushi et al. 2008; Gysels, Pell et al. 2009). The latter indicates the importance of providing, in addition to the medication, clear, relevant information.

Respondents' concerns about the care that their infants would receive when the trial ends highlight the broader issue of the feasibility of implementing IPTi in PNG and in other countries with resource-poor health systems. Research in sub-Saharan Africa has, however, shown IPTi to be a cost-effective intervention (Conteh, Sicuri et al. 2010) and pilot implementation studies undertaken by UNICEF in six African countries indicated that IPTi with SP was a feasible intervention, reaching 97% of immunized infants (de Sousa, Salama et al. 2010). In spite of these, findings, efforts are required to address the poor coverage and timeliness of childhood immunization outside of the urban centres in PNG (Toikilik, Tuges et al. 2010). Furthermore, in a third of cases reported in this study, non-vaccination was attributed to problems with the health services (Toikilik, Tuges et al. 2010), which illustrates the need for investment in the provision of MCH outreach services. Although, the data presented provide some insight into health workers' behaviour towards mothers attending MCH for immunization and its effect on immunization adherence, this requires further in-depth study.

The data collected in PNG illustrate additional, striking similarities to those from acceptability studies undertaken in sub-Saharan Africa. For example, mothers in all sites were familiar with vaccination as prevention yet uncertain regarding particular diseases prevented and the nature of the prevention. Also the key barriers to attending clinics, mainly distance and transport and treatment costs, were common in sub-Saharan Africa and PNG. There were also common complaints about (non-trial) health staff that criticize and chastise mothers in the clinic (Schwarz, Gysels et al. 2009). The data also suggest that the role of fathers in infant care is similar: although fathers play a relatively minor role in day-to-day childcare, when more serious matters arise, such as participation in a clinical trial or severe illness, they take the role of decision-makers. Even the nature of the rumours that circulated around the clinical trial of IPTi in PNG, relating to the selling of the blood sampled, resonated strongly with the rumours encountered in other trial sites (Pool, Munguambe et al. 2006; Gysels, Pell et al. 2009).

Strengths and limitations of the study

A clinical trial is not the ideal context to investigate the acceptability of any intervention as many of the factors that affect a community's response to the intervention itself are influenced by the presence of the trial. However, in the case of IPTi in PNG, it was the only way of collecting acceptability data early enough to be able to make recommendations for improving access and long-term adherence during roll out. Using a range of methods (including direct observation of behaviour) and interviewing a range of respondents, enabled the triangulation of findings and ensured their reliability.

7.6 Conclusions

The findings presented complement a broader programme of social science research undertaken in sub-Saharan Africa (Gysels, Pell et al. 2009). The inclusion of PNG as an additional site for acceptability research enables comparisons to be made between this very different social and cultural context and the sites in sub-Saharan Africa. The results of the acceptability research in PNG are remarkably similar to those from the African sites and this allows tentative generalization regarding IPTi implementation beyond Africa. Firstly, IPTi seemingly fits well with local health cultures and appears to be accepted easily. Secondly, there is little evidence that IPTi has negative impacts on attitudes to EPI or EPI adherence. Furthermore, although in PNG mothers described IPTi as complete malaria prevention this did not, as in other African sites, influence existing malaria prevention and treatment-seeking behaviour. In practical terms, the degree of similarity across the sites suggests that IPTi would require minimal adjustment in order to be rolled out successfully across diverse social and cultural contexts. The generalizability of findings vindicates the overall research approach of undertaking small-scale social science studies in many sites.

Competing interests

The authors declare that they have no competing interests

Authors' contributions

CP analysed the data and wrote the manuscript. LS trained the fieldworkers. LS, SP, IM, PS and NS planned and supervised data collection. SL and LS carried out data collection. MG and RP designed the IPTi acceptability research programme and the data collection tools. All authors provided comments on the draft manuscript and approved the final version.

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8 General discussion

8.1 Rationale

The aim of the present work was to explore the context and potential benefits of implementing an IPTi intervention in PNG. First, it was important to have a good understanding of the epidemiology of malaria in PNG. This was the objective of chapter 3 where the relationship between malaria endemicity and population Hb means & population anemia prevalence was assessed. Second, the management of malaria in Papua New Guinean children was for many years to treat presumptively all patients with fever. In chapter 4, the effectiveness of treating malaria with a unified treatment for *Pf* and *Pv* (artemether-lumefantrine, AL) based exclusively on RDT results was assessed. Third, if the efficacy of the IPTi strategy is well established in African countries with a predominance of *Pf* infections, nothing is known on the potential efficacy of IPTi in regions of the world with a high burden of non-*Pf* infections. This was addressed in chapter 5 were the efficacy of a single dose of SP associated to 3 days of AQ or AS was investigated. Fourth, in order to have a broader picture of the burden of diseases in PNG and how would fit an IPTi intervention in this context, we looked in chapter 6 at the potential benefits of IPTi on the overall morbidity as well as the appropriateness of the IMCI strategy supplemented with RDT to guide malaria treatment. Finally, one of the challenges of implementing any new intervention such as IPTi is to be sure that it is understood and accepted by the targeted population. This is addressed in chapter 7 where a qualitative anthropological investigation of the acceptability of IPTi in PNG was undertaken.

All these different aspects combined give a comprehensive picture of the context and potential benefits implementing an IPTi intervention in PNG.

8.2 Diseases burden in PNG

8.2.1 Respiratory infections

As described in chapter 6, the major contributors to morbidity in PNG are respiratory infections as they are the leading cause of illness episodes in children under 5 years in PNG with almost one episode of lower respiratory tract infection (LRTI) per child per year (syndromic definition: tachypnoea + cough and/or difficulties in breathing). This is followed by malaria and gastro-enteritis with approximately 0.7 episode / child / year each. The age pattern of these different diseases is very different with respiratory infections and gastro-enteritis decreasing rapidly as age increases while malaria incidence is increasing with age, being the higher between 18 and 21 months (see fig 2 & 3, chapter 6).

These estimates based on signs and symptoms, except for malaria, are a bit higher than what was described in other settings, even if the present findings are consistent with another study carried out in the PNG Highlands in the 90', where an incidence rate of LRTI of more than 1 episode/child/ year in children under 5 years was described by Smith et al. (Smith, Lehmann et al. 1991) In a recent observational study in Kenya, incidence rate of 0.2 to 0.6 was reported for LRTI in children below 2 years (Feikin, Olack et al. 2011).

A number of factors could explain a potential overestimation of ARI and LRTI in PNG and need to be considered before concluding that their incidence rates are higher in PNG. First, the definitions used might differ slightly from one place to the other. Indeed, in PNG for example, a distinction is made between mild (fast breathing + cough), moderate (+ chest indrawing) and severe pneumonia (+ danger signs such as tachycardia and cyanosis). Thus, a moderate pneumonia in PNG corresponds to a severe pneumonia in the WHO classification. Second, one of the main components of the definition of LRTI is tachypnoea. However, the estimation of the respiratory rate might vary greatly between observers. More generally, a regular training of health workers is required to achieve good clinical assessment (Horwood, Vermaak et al. 2009). Third, the reporting system of cases might vary greatly from one region to the other. Indeed, in the present drug

trial, parents were strongly encouraged to visit the health facilities, free of charge, when their child was sick. Regular visits to villages to perform the IPTi intervention might have also increased the attendance rate of sick children. However, and even considering these potential reasons for an overestimation of its burden, LRTI and more generally ARI are leading causes of morbidities in young Papua New Guinean, especially below one year.

Several reasons might explain this high burden of ARI and LRTI observed in PNG. First, several surveys performed in PNG showed that *Streptococcus pneumoniae* and *Haemophilus influenza* (Hib) were highly prevalent as a cause of pneumonia (Lehmann 1992; Riley 2002). It is also the only country where a polysaccharide vaccine against *S. pneumomiae* has demonstrated an effect on overall mortality with a decrease of 19%. (Riley, Lehmann et al. 1986) Even if these studies were mainly performed in Highlander populations of PNG which are different from Madang, it is possible that the effect on mortality reflects also a higher susceptibility of Papuan children to this pathogen and could explain therefore why the burden of LRTI is higher compared to other developing countries.

Nutrition factors might also play a significant role in terms of susceptibility to LRTI. Indeed, it was demonstrated that children in PNG and elsewhere with poor nutrition status and low birth weights are particularly at risk of LRTI, especially infants below 6 months. (Smith, Lehmann et al. 1991; Lehmann and Heywood 1996; WHO 2011) Due to a poor diet and unfavourable socio-economic factors, PNG presents very high prevalence rates of children under 5 years with low height-for-age (stunting) and low weight-for-height (wasting) (Mueller and Smith 1999; Mueller, Vounatsou et al. 2001). Pattern of malnutrition is different across the country. In the Highlands, stunting is predominant (>50%) with little wasting, while on costal areas (such as Madang), wasting is more common (3-5%) and frequently associated with stunting (30-40%).(Norgan 1995) We can therefore postulate that these factors contribute to make young children of PNG especially susceptible to LRTI and thus increase the overall incidence. Other environmental factors such as cooking on fire inside the house (indoor pollution), passive

smoking or overcrowding, which are common in PNG, might also contribute to the high burden of ARI/LRTI observed.(Armstrong and Campbell 1991; Smith, Samet et al. 2000; Suzuki, Thiem et al. 2009)

8.2.2 Malaria

For malaria, the story is more complex. Indeed, with approximately 0.7 episode per year, PNG can be considered as hyperendemic according to WHO definition (WHO 2010). This figure, however, does not reflect the local variations of endemicity. The IPTi trial is a very good example. Indeed, originally the study was planned on two sites (Mugil in Madang province and Wosera in East Sepik province) because they were supposed to be equivalent in terms of malaria transmission: same altitude, similar geography & climate (**See figure 8.1**), rural setting, previous report with similar EIR (Mueller, Bockarie et al. 2003). However, during the IPTi trial, very different transmission intensities were observed. Indeed, the incidence of malaria in children aged 3-15 months was extremely low in the Wosera with less than 0.1 episode/child/year (interim analysis of the IPTi study, personal communication) compare to 1.07 episode/child/year in Mugil, 0.28 for *Pf* and 0.81 for *Pv* (placebo group, IPTi trial, chapter 5). It is not totally clear why the endemicity decreased so rapidly in the Wosera from approximately 1.3 episodes/child/year in the early 90' (Shankar, Genton et al. 1999) to less than 0.1 during the IPTi trial (interim analysis, personal communication), but several hypotheses can be formulated. First, a couple of months prior to the beginning of the study, a "wild" distribution of ITN by a non-governmental organization (NGO) was initiated in the region. Knowing the effectiveness of this control intervention, (Lengeler 2004) it is likely that it has contributed to this drop. Second, small climate changes (drier, warmer) might have made the environment less favourable for malaria transmission. It is often difficult to predict, because even if simple climate determinants (rainfalls, temperature) are well recognized factors influencing malaria transmission, more complex ecological characteristics (deforestation, agriculture or water quality) might also influence the local epidemiology of malaria. (Paaijmans, Read et al. 2009; Parham and Michael

2010; Stresman 2010) Finally, the Wosera area has been involved for more than 20 years in various research projects on malaria. This might have been a highly efficient intervention for malaria control (but not much cost-effective!), probably because almost the entire population was sensitized to the problem of malaria and had a better access to malaria interventions than other regions. Apart from the ITN intervention, we are not aware of any other intervention such has mass drug administration that could have taken place in this region.

Figure 8-1: Typical landscapes of both the Wosera and Mugil areas, the original 2 sites planned for the study.

Wosera **Mugil**

This example highlights the fact that local endemicity can vary rapidly over a relatively short period of time. This demonstrates also the complexity of monitoring malaria and the importance of using appropriate tools to measure these variations. In regards to that, it might be important to consider anaemia prevalence or population Hb mean as additional indicators to the known arsenal of metrics such as parasite rate or *Pf* incidence rate. Monitoring malaria transmission variations becomes critical when planning and when prioritizing the implementation of control interventions such as ITN, IRS or even IPTi.

8.2.3 Gastroenteritis

Diarrheal syndromes were frequent reasons for OPD visits in our cohort with an overall incidence of 0.7 episode/child/ year. These findings are in line with what was found recently in Kenyan infants, where incidence rates of 0.4 to 0.7 were described in children under 5 years.(Feikin, Olack et al. 2011)

However, these figures are much lower than what was observed in PNG previously. A study performed in the late 80' described a much higher burden with around 5 episodes per year in the Highlands of PNG in the same age group.(Wyrsch, Coakley et al. 1998) WHO reported various incidences from developing countries, but generally, they are well over one episode per year, even in the Asia-Pacific areas, except Indonesia where an incidence of around 0.8 was reported in the late 80'. (Kosek, Bern et al. 2003) There are only few recent estimates of incidence of diarrhea in developing countries. However, all indicate an important decrease of its burden. Reasons are not all well established. Main factors that might have impact on diarrhea are: better living conditions, better hygiene and better awareness. It is also likely that the reporting methods were different and diarrheal episodes were defined differently in the past.

8.2.4 Acute otitis media (AOM)

Surprisingly, acute otitis media were much less frequently diagnosed compared to other syndromes. It is also much lower than elsewhere. Indeed, only 2.8% of cases seen in the study clinics had a diagnosis of AOM, which is 10 times less than what was reported in Nigeria in a small survey, where 29% of children under 5 years were diagnosed with AOM in an outpatient clinic.(Amusa, Ogunniyi et al. 2005) It is also very low compared to what is reported in developed countries, where incidence rates of 1.7 to 1.9 episodes/child/year are reported in children below 2 years (PNG = 0.06-0.09).(Chonmaitree, Revai et al. 2008; Rovers 2008) It is especially surprising considering that OAM usually follows the occurrence of upper respiratory tract infections (URTI). As we have seen, URTI are very common in PNG, but seems not to be associated with a high burden of AOM.

Reasons for this very low incidence are not entirely clear, but it is likely that AOM are under diagnosed because of difficulties for the routine health staff to perform good quality otoscopies. Moreover the diagnosis is often made only when pus is discharging from the ear, which is a sign of a perforated AOM corresponding to a minority of all cases. Finally, it is now well established that most AOM heal spontaneously without any antibiotics. (Coker, Chan et al. 2010) AOM could therefore remain undiagnosed without having important consequences for the child.

8.2.5 Burden of diseases and public health implications

These data show clearly that ARI and LRTI are leading causes of morbidities in Papua New Guinea infants. If important resources have been allocated to better understand and control malaria in the recent years in PNG, it seems also urgent to plan interventions that could impact on the burden of respiratory infections. These interventions should aim first at preventing the occurrence of LRTI and their complications. In this regard, the recent introduction of *Hib* vaccine in PNG will certainly have a positive impact on the burden of respiratory infections as it was shown in resources limited countries. In a second step, the introduction of the immunization against *S. pneumoniae* could be also considered. (Gessner 2009) Malnutrition, among other factors, increases the risk of respiratory infections, especially in infants. Therefore, interventions should also emphasize the importance of integrating correction of malnutrition. The same applies to other co-factors such as indoor air pollution where prevention should be reinforced. Finally the epidemiology of respiratory infections in PNG, especially in regards to aetiologies, is still unclear and should be investigated in order to improve treatment and prevention strategies.

The high burden of malaria is also an important problem in PNG. As it will be discussed below, it will be important to measure the impact of the recently introduced prevention strategies such ITN and ACT to prioritized further interventions.

Diarrheal syndromes seem to have considerably decreased in the past 20 years, which is good news for health authorities. Simple sanitation measures account certainly for an important part of this drop and efforts should be maintained to promote a better hygiene and safe access to water.

8.3 Main findings about IPTi in PNG

This work provides the proof of principle that IPTi using a combination of two long acting drugs (SP associated with 3 days of AQ) reduced the risk of malaria (all species) by 28%. On the other hand, SP associated with 3 days of AS (a short acting drug) had only a limited effect on malaria. SP-AQ3 worked better against *Pf* than *Pv* (ITT 37% vs 22%, ATP 51% vs 31%). It reduced the risk of anemia (<8g/dl) by 25% (non-significant in ITT analysis). It had no effect on the overall morbidity (RR=1.0, p=0.99), no effect on serious adverse events (RR=1.11, p=0.84) but a surprisingly lower risk of death (7 in the placebo group, 2 in the SP-AS3 group and none in the SP-AQ3 group). Resistance levels for both SP and AQ were high in the study area, however different for *Pf* and *Pv*.(Marfurt, Mueller et al. 2007) Indeed, SP is mainly effective against *Pf* while AQ is much better for *Pv*. This explains why this drug combination (SP-AQ3), even though suboptimal, is complementary in preventing malaria in PNG, highly endemic for both species. No serious adverse event related to the study drugs was observed.

Nevertheless, these encouraging efficacy results are only a first step on the long way leading to a successful implementation. The feasibility and appropriateness of such an intervention is complex and depends on a lot of different factors. Indeed, several factors can be identified that could compromise the benefits of the IPTi intervention when implemented in routine practice, which refers to the effectiveness. These aspects will need to be considered when health authorities might decide on IPTi policy in PNG. Therefore, the following **section 8.4** will detail the main determinants that could affect the IPTi effectiveness, i.e.

- **the EPI coverage**: a key component for IPTi delivery

- **The endemiciy of malaria in PNG**: IPTi is beneficial mainly in areas with moderate to high endemicity. This might change rapidly in PNG due to the recent introduction of control strategies (ITN and ACT).
- **The effect (or the absence of effect) of IPTi on the overall morbidity:** This is important to consider from a public health perspective, because it will necessarily impact on the health resources needed
- **Drug combinations:** The IPTi trial investigated two different drug regimens that are not perfect in many aspects such as resistance and safety profiles. Therefore, it is important to consider whether other drug combinations might be useful to investigate.
- **Acceptability:** This aspect of the intervention is also important and was investigated along side the trial.
- **Feasibility:** This includes, among others, the availability & delivery of the drugs, the adherence to the home delivery of drugs and the ease of administration of the drugs
- **Cost-effectiveness:** This is essential to consider from a public health perspective.

All these factors should be considered one by one for their **implications for policy decision**.

8.4 The determinants of IPTi effectiveness in PNG

8.4.1 IPTi coverage depends on EPI coverage

One of the main features of IPTi interventions is to use as much as possible the existing health infrastructure. Practically, the trial was set up in collaboration with local health facilities responsible to conduct the EPI in their own catchment areas. The study teams joined the routine staff during outreach clinics and performed the IPTi study while nurses from the health centres were taking care of the immunizations as well as of the MCH clinic. Therefore, if one would like to implement IPTi in PNG, logistics is not to be one of the main barriers as no

supplementary infrastructures will need to be set up. This is one strength of the IPTi intervention compared to other types of intermittent preventive strategies such IPTc which are stand-alone strategies. The feasibility issue has been mentioned in several publications (Beeson, Rogerson et al. 2011; Bojang, Akor et al. 2011) and specifically studied for IPTi in an effectiveness trial performed in 2006 in Tanzania (Willey, Armstrong Schellenberg et al. 2011). They concluded that IPTi implementation is feasible using existing health facilities at limited cost.

One of the key determinants of the effectiveness of IPTi is the level of coverage (proportion of children receiving the intervention in real-life settings). Indeed, to achieve a reasonable effectiveness, it is important that most of the children get the desired four IPTi doses. In the present trial, infants who received at least 3 doses were better protected compared to those who had less doses (38% against all species if the infants get at least 3 doses vs 28% in all infants). Similarly, an important difference of efficacy was observed between intention-to-treat and as-to-protocol analysis, which also reflects the relation of coverage to effectiveness.

Usually, the DPT3 vaccine time point is used to estimate the vaccine coverage and to extrapolate potential IPTi coverage (Carneiro, Smith et al. 2010 Nov; Willey, Armstrong Schellenberg et al. 2011). The only available data on IPTi coverage in routine practice come from Tanzania, where they observed that 72% of children received at least 1 IPTi dose and only 22% received all 3 scheduled doses. The vaccine coverage ranged from 69% for DPT2 (IPTi-1) to 42% for measles (IPTi-3). (Willey, Armstrong Schellenberg et al. 2011) In PNG, vaccine coverage is rather low, with similar figures as Tanzania, around 50-60% for DPT3 and measles 1 (at 6 months of age, IPTi dose 2) to less than 50% for the second shot of measles at 9 months (IPTi dose 3). Figures are not known for the last EPI time point, when infants receive the second dose of vitamin A supplementation (and last dose of IPTi), but it is believed to be very low. We can thus assume that IPTi coverage is likely to be low, which should affect directly its effectiveness. This is below the minimal GAVI recommended cut-off of 70%

DTP3 coverage required for any new intervention to be implemented within EPI (which usually applies to highly effective vaccine). (GAVI 2011)

It seems therefore of prime importance that, if IPTi is considered for implementation, health authorities consider improving EPI coverage. It is also unlikely that the introduction of IPTi by itself could improve the coverage of EPI. It seems thus not straightforward that with about 30% of efficacy in a highly control trial a measurable impact of IPTi on malaria could be observed in real-life condition with such a low coverage.

8.4.2 Changes in transmission intensity of malaria in PNG

As several studies pointed out, the effectiveness of IPTi is increasing as malaria endemiciy of malaria increases. (Kobbe, Adjei et al. 2007; Carneiro, Smith et al. 2010 Nov; Ross, Maire et al. 2011) These studies highlighted also the need to monitor closely the endemicity of malaria in order to proper implement IPTi. This is another challenge that health authorities will have to face, would they wish to implement IPTi in PNG. Indeed, we have seen above how endemicity of malaria can change rapidly and almost unnoticeably between two regions (Wosera and Mugil). On top of this, very effective interventions such as long lasting insecticide impregnated nets (LLINs) and standard treatment with ACT start to be implemented at scale in PNG, which is going to greatly affect the endemicity of malaria all over the country. In such circumstances, it might be difficult: 1) to identify the regions that could really benefit from an IPTi intervention 2) to measure the impact attributable to IPTi.

8.4.3 Effect on overall morbidity, severe illnesses and mortality

One fundamental aspect of any public health intervention is to improve the health status of individuals. It is thus important to measure the impact of an intervention on the overall morbidity, severe illnesses and mortality (on all illness episodes) as it was described by Murray et al. (Murray and Chen 1992), and as it was reliably done for ITN. (Lengeler 2004) In the case of IPTi, apart from the effect of

the intervention on malaria related morbidities (malaria episodes, anaemia), the outcomes of interest are the outpatient attendance rates (overall morbidity), hospitalisation/severe illnesses rates and mortality.

In the previous eight trials described in table 2 (chapter 1), six found no effect on the outpatient visits rate or did not reported it. One reported a limited impact within one month after IPTi dose 1 and 3 in Ghana (OR= 0.80 [0.68 to 0.96] after dose 1 and OR= 0.71 [0.59 to 0.84] after dose 3) and one trial in Mozambique reported an impact on outpatient attendance with chest indrawing within one month after dose 1(RR=0.57, p=0.025). (Chandramohan, Owusu-Agyei et al. 2005; Macete, Aide et al. 2006) None reported a positive impact on the overall outpatient attendances during the first year of life. Similarly for hospitalisations/serious illnesses, two found a significant impact of 19 and 30%, (Schellenberg, Menendez et al. 2005; Macete, Aide et al. 2006), one found a limited impact one month after dose 1 (Mockenhaupt, Reither et al. 2007) and one found an impact on anemia-related admission only. (Macete, Aide et al. 2006) Looking at the present IPTi trial in PNG and as described above, no effect was found on overall morbidity during the first year life, neither on hospital admissions/severe illnesses. Surprisingly, an effect on mortality was found, even though the trial was not designed to assess this outcome. None of the other trials measured any impact on mortality.

The findings presented here tend to demonstrate that the reduction of the malaria incidence in the treatment arms is not accompanied by a reduction of the overall morbidity. Similarly to all other trials performed in African countries, the study was not aimed at investigating the effect of IPTi on overall morbidity. The lack of effect on all episodes in spite of a 30% reduction of malaria episodes means that malaria episodes have been replaced by another (other) disease(s). There was no trend for incidence of all ARI or GI to be higher in the treatment arms. However, there was an indication that severe LRTI could be more frequent in those who received IPTi (IRR for SP-AQ3=1.38, p=0.18). Outside being due to chance, this observation suggests that an interaction might exist between malaria and respiratory infections, malaria providing some protection against some types

of ARI. This is rather contraintuitive, the hypothesis being more for an aggravation of respiratory infections in the presence of malaria parasites. In a recent trial on efficacy of the RTS,S/AS02D malaria vaccine, children receiving the vaccine had significantly less pneumonia compare to the placebo group (receiving hepatitis B vaccine). (Abdulla, Oberholzer et al. 2008) It was never clear if this was an effect due to the decrease of malaria (disease interaction) or a protection due to the vaccine via the adjuvant boosting a non-specific immunity. In the IPTi trial in PNG, the tendency for severe pneumonia to be more frequent in the treatment arms would be in favour of the second hypothesis as we observed more pneumonia in the treatment arms (where malaria incidence was lower).

It is unlikely that side effects du to the study drugs could have “filled the gap”. Indeed, the safety profiles of the study drugs used are well known and recent data from Africa confirm that no more side effects are observed in children who received these drugs compared to other treatments of placebo. (Bojang, Akor et al. 2010)

Part of the explanation could be attributed to co-infections. Indeed, in chapter 6, it is described that about 20% of children with a confirmed diagnosis of malaria (based on RDT) have overlapping signs and symptoms with LRTI or gastroenteritis. This would mean that by preventing malaria parasitemia with IPTi, we still don’t get rid of some other co-infections, which are the real causes of outpatient attendances. This raises the important question of defining “malaria disease” and its use as outcome in drug trials.

If the IPTi trial in PNG did not show protection neither against overall morbidity nor against severe diseases (malaria-related or not), it showed a surprisingly protective efficacy on all-cause mortality. The effect on mortality is important and could justify by itself on deciding to implement this intervention in PNG. This finding needs however to be taken cautiously for several reasons. First, none of the Africans trial has shown any effect on mortality. Second the number of deaths is limited (9 during the intervention period, 13 over the entire study period). Third,

as shown in **table 8.1**, it is likely that most of the children died of other diagnoses than malaria in the placebo group; making less likely the hypothesis of a protection against mortality of the IPTi (no effect against other diseases has been shown). Fourth, if an effect attributable to the intervention is observed on mortality, we would expect to find at least a trend for severe illnesses to be less frequent in the treatment arms (a reduction of all-cause hospital admissions was observed in some African trial using SP alone [23% reduction], but not in other trials using alternative drug combinations). Finally, the study was neither designed nor powered to identify any effect on mortality. For all these reasons, it is possible that this result, even tough statistically significant, might have occurred by chance and should therefore not be used as argument to implement IPTi in PNG.

Table 8-1: Death review based on medical records and verbal autopsies

	Arm	number of days since previous visit	Age at death (Months)	place of death	malaria status	Likely diagnosis	comments
1	AQ-SP	50	22	home	unknown	**TB with neurological involvement (paraplegic)**	
2	AR-SP	4	14	home	Neg	**LRTI**	
3	AR-SP	304	20	Health Centre (night duty)	unknown	**unknown**	verbal autopsy at H/C: severe dehydration +/- malaria
4	AR-SP	61	7	home	unknown	**possible malnutrition**	At last visit was wasted (at 60 percentile) with oral thrush. Twin
5	PLACEBO	n/a	4	home	unknown	**LRTI**	Based on verbal autopsy
6	PLACEBO	7	7	hospital	Neg	**Meningitis**	Already admitted for LRTI for 10 days and developed meningitis
7	PLACEBO	25	4	home	unknown	**Severe Bronchiolitis**	Based on verbal autopsy
8	PLACEBO	2	6	hospital	Neg	**Severe respiratory infection**	
9	PLACEBO	1	4	home	Pv (RDT + BS pos)	**Severe malaria with severe anemia +/- severe respiratory infection**	Treatment with Co-artem started on prvious visit
10	PLACEBO	35	unknown	home (outside province)	unknown	**unknown**	
11	PLACEBO	15	9	home	unknown	**Severe Dehydration**	Verbal autopsy: severe diarrhoea, pallor and fever)
12	PLACEBO	1	14	Hospital	Neg	**Severe Gastro-enteritis with dehydration**	
13	PLACEBO	1	4	Hospital	Pf (RDT pos & BS neg)	**LRTI (vs malaria)**	Patient was treated for malaria 10 days before (RDT=pos Pf)

Even if the reasons for an absence of benefits of IPTi on the overall morbidity are not totally clear, this important observation calls for some comments:

- It is highly surprising that none of the trial's publications and even more none of the reviews on IPTi, including an extensive letter report by the Institute of Medicine(IOM 2008), raised the problem of IPTi having no impact on the overall incidence of diseases during the first year of life.

- An intervention that has no impact on overall morbidity and severe illnesses is not going to provide any benefits to the health system, since the workload will be same or even increased because of the intervention.
- When designing a study investigating the impact of an intervention such as IPTi, it is important to carefully choose the outcomes of interest, not only malaria-related, but also on the overall morbidity, including severe diseases or all-cause mortality.
- It is questionable to consider IPTi as a control tool when no effect on morbidity is observed. More generally, when countries are embarking on malaria control programs, it is essential to measure their impact on non-malaria related outcomes as well.
- To confirm these findings from PNG (and the rare published information from African trials), it is urgent to perform again pooled analysis of all available data from IPTi trials and to look at the overall and non-malarial morbidities
- Policy makers in PNG should consider the absence of benefits of IPTi on overall morbidity before to decide on a potential implementation of the intervention

In summary, these observations raise important concerns about the real benefits of the IPTi intervention in PNG, especially when it should be used as a control tool for malaria. Indeed, which country would like to implement a malaria control intervention that does have no impact on the overall morbidity or on severe diseases? Additionally, it might be difficult to explain the “IPTi paradox” (efficacy against malaria, no impact on overall morbidity) to parents, see **figure 8.2**.

<u>Figure 8-2</u>: Translation in simple words: the IPTi paradox explained by a nurse to a mother visiting the EPI clinic in Madang province (PNG).

8.4.4 The choice of the drug combination

The drug combination including two rather one long acting drugs was the most effective to reduce malaria, confirming findings from Africa that it is the post-treatment prophylactic effect that counts. However, in the case of IPTi in PNG, these two drugs (SP and AQ) are rather imperfect. Indeed, high levels of resistance against *Pf* are documented with AQ (high prevalence of fixation of the SVMNT *pfcrt* haplotype and *pfmdr1* 86Y mutation) and SP with *Pv* with high prevalence of quadruple *pvdhfr* mutation (see chapter 5). These data are in line with previous findings by Marfurt *et al* that high levels of resistance of *Pf* and *Pv* to SP-AQ3 are observed in Madang province.(Marfurt, Mueller et al. 2007; Marfurt, de Monbrison et al. 2008; Marfurt, Muller et al. 2008) Few trials have investigated other long acting drugs, alone or in combination, but the only that has shown encouraging results is mefloquine even if its tolerability was poor. (Gosling, Schellenberg et al. 2006) The main issue with SP, apart from resistance, is the potential of severe side effects (Steven-Johnson Syndrome). Therefore if one would like to pursue with IPTi and investigating alternative regimens, research should focus on long-acting drugs, well tolerated and with a favourable resistance profile. Not many drugs fulfil these criteria, but the recently developed combination of dihydroartemether /piperaquine (DP) might be a good

candidate. Piperaquine is a bisquinoline having one of the longest half-life of all antimalarial and is active against *Pv*.(Karunajeewa, Mueller et al. 2008; Awab, Pukrittayakamee et al. 2010) At present, a trial is going on in Burkina Faso to investigate the efficacy of DP used as seasonal IPTc (ClinicalTrial.gov register number: NCT00941785). It is still questionable to use ACT for IPT interventions when these efficient treatments are also used as first line therapies in the country as this might contribute to speed up the level of resistance. Furthermore, it is probable that short acting drugs such as artemisinin derivates do not contribute much to the overall protective efficacy of IPTi. On the other hand, adding a highly efficient drug that will rapidly treat existing blood stage parasites might help to protect the efficacy of the associated long acting drug. This question remains open.

However, if a new and more efficient drug regimen is investigated, it will be important to consider also the impact on the overall morbidity. In line with this, some research group are looking at the efficacy of broad spectrum antibiotics with antimalarial activities (such as azitromycine) on malaria and non-malaria outcomes in pregnant women. However, using antibiotics as preventive mass drug administration raises major issues though in terms of emergence of drug resistance in countries who cannot afford expensive alternatives.

8.4.5 Acceptability

One key aspect when planning to introduce a new intervention such as IPTi is its acceptability. Indeed, beside the efficacy itself, it is important that parents who are going to give the drugs to their child agree with it. This was investigated in Africa and acceptability was found to be very good.(Pool, Mushi et al. 2008) The same team investigated the acceptability of IPTi in PNG, because the social and health context is completely different from Africa. Similar reassuring results were found in PNG, where IPTi seems to be well understood and accepted by the care takers.

8.4.6 Feasibility of IPTi in PNG

IPTi has been conceived to be easily implementable since it uses the EPI delivery platform. Access should not be an issue if EPI clinics are well organized. EPI coverage in PNG is low (about 50%), which will affect the delivery of IPTi, and hence its effectiveness. Appropriate training of staff is also important. But, as this intervention is very simple (giving a malaria treatment course to infants coming for immunization), a minimal training of 1-2 days is sufficient to achieve a good understanding of the intervention by health staff and a correct delivery of treatment (which was more complex than it would be in routine practice because of the trial constrains).

The nature of the intervention implies that parents administer themselves the second and third dose of treatment (AQ). This was a concern and non-adherence to the 2nd and 3rd doses was feared. We established a community reporters' network which was responsible to record if parents gave the drugs. According to their reports, it appears that adherence was good with more than 90% of treatment given. To better document these observations, drug levels have been measured in a random sample of 200 children on the day following the last IPTi dose given by parents. Analyses of data are still ongoing and results will be available in a near future. But according to preliminary results, it appears that drugs levels were high in all treatment groups (except placebo).

The availability of the drugs used is also important. But this was not a problem as the treatments used in the PNG trial were SP, AQ or AS which were all first line treatments in the country at the time of the trial. The only concern might be related to the quality of the administration. Indeed, no infant formulation (syrup or granules) does exist for any of the drugs that were used, which could make their delivery more complex. It has been shown in a recent review that infant formulations of antimalarial drugs improve the uptake of the treatment. (Kurth, Belard et al. 2010) In the present trial, tablets were crushed, mixed with syrup and administered with a spoon or a syringe. This procedure was acceptable but could be much more satisfactory if infant formulations would exist. The NGO Medicines for Malaria Venture (MMV, www.mmv.org) is committed to promote

the development of such convenient drug formulations, which would render IPTi easier to implement and eventually more effective.

In summary, IPTi in PNG seems feasible, similarly to what was shown in Tanzania (Armstrong Schellenberg, Shirima et al. 2010; Willey, Armstrong Schellenberg et al. 2011), even if the EPI coverage is certainly the strongest limitation.

8.4.7 Cost-effectiveness of IPTi in PNG

Because of the limited resources, many interventions in developing countries are evaluated for their usefulness as public health tool through the prism of cost-effectiveness. IPTi makes no exception to this, and it was extensively studied in Africa. Two studies investigated the cost effectiveness of IPTi using SP, AQ3-AS3 and mefloquine. The cost per malaria episode averted ranged from 1.4 to 4.0 US$ with SP used alone. It was 4.7 US$ for AQ3-AS3. The intervention was considered to be highly cost-effective in most of the studies with 2.9 to 39.6 US$ per DALY averted. (Hutton, Schellenberg et al. 2009; Conteh, Sicuri et al. 2010) Another study developed a model to estimate the cost-effectiveness of IPTi with SP or AQ3-AS3 according to specificities of different settings and concluded that IPTi with these drugs is likely to be cost-effective in settings with high transmission intensities, good implementation and “not too high” resistance levels. (Ross, Maire et al. 2011) The figures for PNG are not known even if a study has been carried out, but results are not yet available. It is however likely that conclusions might be similar to what was already published.

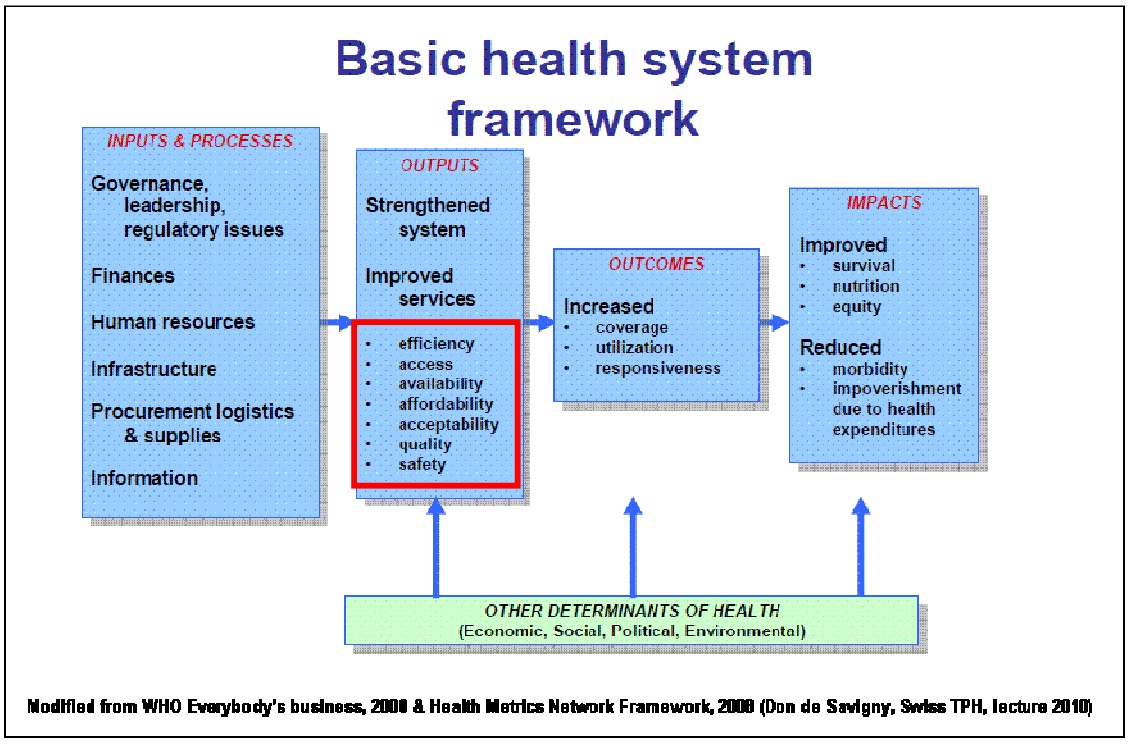
This approach, focused on malaria outcomes, could be however criticized. Indeed, all these studies are assuming that reducing the burden of malaria will also reduce the overall burden of diseases (DALY’s are not related to a particular disease). This was already highlighted by Donaldson *et al* in the British Medical Journal in 2002, where they warned against the danger of choosing the wrong efficiency question. (Donaldson, Currie et al. 2002) In the case of the IPTi trial in PNG, no impact was detected on the overall morbidity measured as the incidence of outpatient attendances (this was extensively discussed above). It is

not very clear why no effect was observed on the overall morbidity, but it seems however reasonable to integrate this aspect into the model assessing the cost-effectiveness of IPTi. Thus, an intervention that has no impact on the overall morbidity or on severe illnesses is by definition only costly.

8.4.8 Implications for policy decision

On the long pathway towards moving health interventions from innovation, to validation, policy making and application, several aspects need to be considered. **Figure 8.3** displays a general diagram of health system framework.

Figure 8-3: Basic health system framework summarizing the key features of a health system.



Many aspects presented in figure 8.1 are directly related to policy decision. At the present stage of IPTi development, the items in the red frame are especially interesting in regards to policy decision. 1) The *efficiency* of IPTi has been

extensively described above and concerns have been raised about the overall benefit on morbidity and severe illnesses, while the intervention significantly decreased the burden of malaria. 2) The four next items *accessibility*, *affordability*, *availability* and *acceptability* refer to an access model developed by Penchansky and Thomas in the 80' (Penchansky and Thomas 1981) and was further developed by other health researchers. (Obrist, Iteba et al. 2007) In the present IPTi trial, *acceptability* was good (see chapter 7). Both *affordability* and *availability* are also not much of a concern, as the drug regimen proposed included SP and AQ, which are cheap and available in PNG even if their availability is strongly dependant on the EPI coverage. *Accessibility* is likely to be the most challenging, also because of the low coverage of EPI. Finally, *quality* and *safety* are not important barriers to IPTi implementation as it was showed in the trial.

In addition to all these points, there is still a controversy on the role of IPTi in the panel of strategies to fight malaria. Indeed, if some experts consider IPTi as a useful additional control tool (in conjunction with LLINs, IRS and ACT-based therapies), (Aponte, Schellenberg et al. 2009), for others, it is simply a way to improve malaria case management by reducing the incidence of severe illnesses without having a significant impact on malaria transmission. (Aguas, Lourenco et al. 2009) All these expert opinions refer to the African situation. What should be considered for the PNG context? It is probably more complex as the endemicity is very different from Africa, being high for both *Pf* and *Pv*. As it was described all along this thesis, PNG is still highly endemic for malaria and no important drop was observed compared to what has been seen in several African countries. PNG is considered to be in the control phase, according to WHO definition (WHO 2010), and rather far away from embarking on an elimination program. The main reason is that control strategies such as LLINs, IRS and ACT-based treatments have just been recently implemented and it is too early to measure any impact of these interventions on the burden of malaria. In such a context of rapidly evolving endemicity, it might be hazardous to introduce simultaneously an additional intervention that did not show a clear effect on overall morbidity and that is not

meant to have a significant effect on malaria transmission. A strong effort should rather be put on achieving high coverage of recently introduced interventions.

In summary and from a public health perspective, it might be not recommended to introduce IPTi for the time being in PNG despite its proven efficacy against malaria. The main reasons are 1) no impact on the overall morbidity is expected, 2) with the current reported EPI coverage, the effectiveness of IPTi on malaria is likely to be marginal, 3) in a phase of multi-strategies introduction (LLINs and ACT), it might be wise to achieve high coverage of existing effective interventions instead of adding another one that is unlikely to be cost-effective. Table 8.2 tries to summaries pros and cons for deciding on the establishment of an IPTi policy in PNG.

Table 8-2: pros and cons for deciding on the establishment of an IPTi policy in PNG

Determinants of effectiveness	Pros ….. Cons					comments
Efficacy						
overall morbidity						no effect
all malaria episodes						30% reduction, mainly on *Pf*
anemia < 8g/dl						41% reduction in ATP analysis only
all severe diseases/admissions						no effect
malaria-related severe diseases						no effect
all-cause mortality						Cave: sole trial with efficacy on mortality
EPI coverage						max 50% expected
Choice of the drug (SP-AQ3)						high resistance levels
Malaria endemicity						endemicity changing rapidly (ITN, ACT)
Feasibility						low because EPI coverage is low
Adherence to treatment at home						tested, good in trial setting
Access						
Acceptability						tested, excellent in trial setting
Availability						strongly related to EPI coverage
Affordability						Cheap intervention
Accessibility						strongly related to EPI coverage
Cost-effectiveness						
all morbidity						
malaria-related morbidity						Highly cost-effective in African settings

8.5 Clinical management of malaria and other diseases in PNG

Chapters 4 and 6 investigated the management of childhood illnesses in outpatient facilities. Even if these aspects are not directly linked to the IPTi intervention, they cannot be ignored in the overall context of malaria intervention evaluation. Indeed, it is now well recognized that a combination of efficient strategies is required to achieve significant reduction of the malaria burden. Apart from preventive interventions such as LLINs and IRS, accurate diagnosis of malaria and efficient treatment is also mandatory. (WHO 2010) If the benefits of using RDT and ACT therapies are well established in Africa where *Pf* is the predominant species, it is not the case in PNG where *Pv* accounts for more than half of the burden of malaria. RDT have shown to be less sensitive on *Pv* than on *Pf* (WHO 2008). Similarly, in settings of well controlled clinical trials, ACT's were found to be less effective on *Pv* than on *Pf.* (Karunajeewa, Mueller et al. 2008). These observations called for a careful assessment of medical practices in this context of different epidemiology and suboptimal tools. Chapter 4 & 6 investigate these key aspects of the management of malaria: what is happening in real-life outpatient clinics if infants are treated with AL based solely on the result of the RDT alongside the IMCI guidelines?

8.5.1 Safety of RDT & utility within IMCI

The observations made in the present project showed that, even if there are imperfect to detect low *Pv* parasitemia, malaria treatment guided by the use of RDT is safe in infants in PNG routine practice. Indeed, no case which might have gone to severe malaria or death (out of almost 4000 negative tests results) was missed with this procedure. The recent development of RDT more sensitive for *Pv* is an additional reassurance in this regards. (Maltha, Gillet et al. 2010; Singh, Shukla et al. 2010) This work is in line with findings from Africa, where the safety of withholding antimalarial when RDT was negative was confirmed for *Pf* infections. (d'Acremont, Malila et al. 2010)

The present work allowed also investigating how the introduction of RDT could fit in and potentially improve the PNG treatment guidelines (IMCI). This was done in chapter 6 were it was shown that the use of RDT within IMCI improved the accuracy of malaria diagnosis by health staff with a correlation of 0.99 (kappa) between the diagnosis posed by the clinicians and a standardized diagnosis generated by computer (based on recorded signs/symptoms). On the other hand, the result of the test significantly changed the antibiotics prescription's habits of health care workers as 56% of children received antibiotics when RDT was negative compare to 16% only when it was positive (excluding all other diagnosis requiring the prescription of antibiotics). It is not entirely clear how RDT results influence the prescription of antibiotics: if it decreases it when it is positive or if it increases it when the RDT result is negative (or both). However, it seems important to provide clear recommendations to health workers on the management of children with negative RDT.

These data provide solid evidence that RDT's used in the frame of IMCI are safe and accurate for the management of malaria in young children in PNG.

8.5.2 Safety and effectiveness of artemether-lumefantrine for *Pf* and *Pv*

With the emergence of chloroquine-resistant *P vivax* strains and the risk of misdiagnosis (*Pf* instead of *Pv* or mixed infections), especially in settings such as PNG with high mix-endemicity for both species, having one treatment efficient on all species, a so-called unified treatment, would be welcome and is advocated by some experts. (Douglas, NM. et al. 2010) However, if one trial has investigated the efficacy of ACT on *Pv* in PNG, (Karunajeewa, Mueller et al. 2008) none have looked at the effectiveness of these treatments in routine practice. This is what was done in chapter 4, where re-attendance rates of young children with positive RDT result and treated with AL irrespective of the species were investigated.

AL appears to be a safe drug in the Melanesian population as no severe AE related to the drug was reported. The results on efficacy of AL on both *Pf* and *Pv* are very reassuring. AL showed to be equally efficacious to clear initial parasitemia for both *Pf* and *Pv.* Indeed, the re-attendance rates within 28 days for same species (uncorrected for new infections, recrudescences and relapses) were around 1%. The main issue that remains is to known how to deal with late *Pv* treatment failures (9% of re-attendance by day 42 for *Pv* compare to 3% with *Pf*), mostly due to liver stage parasites not affected by AL. One option could be to add a single dose of primaquine, the only efficient drug against dormant forms of the parasite, as it was proposed by several experts. (Douglas, NM. et al. 2010; Smithuis, Kyaw et al. 2010). This raises a safety concern due to the risk of haemolysis in G6PD deficient patients.(Shekalaghe, ter Braak et al. 2010) Experts recommend systematic testing for enzyme deficiency to be performed prior to treatment, even if the effect of primaquine on red blood cells is dose-dependant. (Moonen, Cohen et al. 2010) Further studies are thus needed to clarify both the effectiveness and the safety of this strategy in PNG. On the other hand, in a country highly endemic for *Pv*, it is questionable to know if it is worthwhile, at an individual level, to treat a patient for hypnozoites if the risk of being re-infected quickly is high. It is however clear that in terms of controlling parasites' transmission countrywide, treating these liver-stage forms is essential (Wells, Burrows et al. 2010)

In a context such as PNG, introducing a unified treatment of AL has some advantages and disadvantages. Among the main advantages, having one single treatment for all species clearly simplifies the task of health workers in routine practice. Because the resistance to chloroquine (or amodiaquine) is common in PNG, (Marfurt, Mueller et al. 2007) the use of AL is safer in case of missed *Pf* diagnosis or mixed infection. However, there are also some drawbacks compared to the use of a differential medication. Indeed AL is more expensive than chloroquine or amodiaquine. If AL is used to treat all malaria episodes, this strategy will be more costly than a dual treatment. The other main issue that

remains unsolved with a unified treatment is the problem of hypnozoites that are not cured with AL. This was discussed in the previous paragraph.

Therefore, from our data and in absence of a better treatment alternative, AL could be recommended as first line unified treatment in PNG.

8.5.3 Feasibility of using RDT-based unified treatment with AL in PNG

The IPTi trial in PNG was a good opportunity to evaluate the feasibility of using RDT and AL in routine practice, even if the trial was not designed for that. This evaluation was timely for PNG since health authorities were discussing on diagnostic and treatment policies.

All nurses using the RDT routinely during the study were working in public health facilities prior to their employment for this trial and were not familiar to the procedure of RDT. At the time of the trial, AL was not a standard treatment in PNG and the nurses never used it before. The effectiveness data detailed in the different chapters of this thesis tend to show that RDT use and AL prescription by the health staff were appropriate and that the treatment was well taken by children.

In terms of staff training, each nurse attended approximately 1-2 days of training on the use of RDT and on the prescription of AL. Additionally, regular on-site quality assessments were performed to ensure that the device was appropriately used. This corresponds more or less to the training provided by the health department to staff in routine clinics (outside trial setting). Informally, staff often reported that they found the new approach towards malaria management better than the previously presumptive treatment. These data confirm findings of many other studies performed around the world, that the implementation of RDT-based treatment is feasible in routine practice (Kyabayinze, Asiimwe et al. 2010; Masanja, McMorrow et al. 2010; D'Acremont, Kahama-Maro et al. 2011)

See **appendix 2** for detailed procedure for malaria in the IPTi trial.

8.5.4 Performance of IMCI on non-malaria illnesses

IMCI supplemented with RDT was detailed above and proved to significantly improve the management of malaria but strongly influenced the prescription of antibiotics, being much higher when the RDT was negative with 56% of antibiotics prescription and only 16% when the test is positive (excluding all other reasons to prescribe antibiotics).

On the opposite, the findings exposed in chapter 6 clearly show that the management of other important syndromes is unsatisfactory, especially for acute respiratory infections: inaccuracy of diagnosis by health workers (K =0.42 for URTI and 0.47 for LRTI), inappropriateness of current guidelines to identify patients with LRTI or gastroenteritis requiring antibiotics (re-attendance rates within 14 days are similar, 8% vs 9% wether children received antibiotics or not with a clinical diagnosis of LRTI [p=0.44]. Rates for gastroenteritis were 9% and 8% respectively [p=0.51]) and inadequacy of antibiotics prescription (25% of children received treatment when they should not or did not receive when they should).

This is especially important for respiratory infections as ARI represent the majority of the burden in Papuan infants (almost one episode of LRTI per child per year below 1 year). It seems therefore urgent to improve the strategy to manage respiratory infections in children. The purpose of the revision should aim at: 1) better identifying children with bacterial LRTI that require antibiotics 2) better identifying children with LRTI that require more intensive care support. These two aspects are complementary and need to be considered together as young children might suffer from a severe LRTI of viral aetiology that requires oxygen therapy and not necessarily antibiotics (ex: complications of a bronchiolitis due to RSV). (Dawson-Caswell and Muncie 2011) If the clinical differentiation between pneumonia and bronchiolitis is relatively easy for experienced clinicians, it is much more difficult for health workers of rural areas not familiar to the use of stethoscope. Following the example of the new strategy developed to manage malaria cases, a potential strategy could be to combine: 1) better clinical definition (increase threshold of respiratory rate and/or include

fever) 2) rapid tests for potential common aetiologies of LRTI and 3) makers of severity of LRTI such as pro-calcitonin, c-reactive protein or the analysis of exhaled gases.(Schmidt, Bhandari et al. 2010) All these measures should both improve the identification of potential severe cases and lead to a more rational use of antibiotics. It is however clear that the problem of management of acute respiratory infections is far more complex than with malaria and it will not be possible to solve the problem by just developing one single diagnosis test as it was done with RDT for malaria. Further research and resources are therefore urgently needed to explore new strategies.

8.5.5 Implications for policy decision of updating IMCI with RDT-based treatment

All the data presented in the different chapters confirm that the use of RDT and AL within IMCI guidelines can be applied in the PNG setting and has the potential to greatly improve the management of malaria in regions highly endemic for non-*Pf* malaria. It seems therefore reasonable to move WHO's health policies forward by updating IMCI and incorporating RDT to guidelines in countries endemic for any malaria species and not only in Africa. On the opposite, it is urgent to improve the guidelines for the management of other diseases, especially respiratory infections. All these changes should be accompanied with a reinforcement of staff training, not only on the use of these guidelines but also on the understanding of their medical meanings.

9 Conclusions

The present work demonstrates that treating young children with artemther/lumefantrine based on RDT results is safe and effective and greatly improves the management of malaria cases in PNG compare to presumptive treatment. This work shows also for the first time that IPTi is efficacious and reduces the risk of malaria by 29% when using SP associated to 3 days of AQ in a region of the world with a highly mix-endemicity for *Pf* and *Pv*. However the IPTi intervention seems not to provide any benefit on the overall burden of diseases or on severe illnesses. This is a concern and mitigates the interest of implementing this intervention in PNG. Furthermore, other factors such as a low EPI coverage and rapidly changing malaria endemicity due to the recent introduction of LLINs and ACT are likely to drastically reduce the potential benefits of IPTi on malaria in PNG.

10 Recommendations and future research areas

10.1 Recommendations for health authorities and policy makers in PNG

Burden of diseases in PNG

- Respiratory infections are the leading cause of illnesses in young children in PNG and their incidence are high compared to other developing countries. Health authorities should consider reinforcing prevention measures that could decrease this important burden. In that regards, the introduction of a vaccine against *S. pneumoniae* should be considered. Programs aiming at improving nutrition status and better living conditions (indoor pollution) should be conducted. These measures should be accompanied by a better training of health staff on the management of ARI and LRTI.
- Measuring the impact of malaria control interventions recently introduced in PNG (ITN, ACT) is essential, especially in the perspective of eventually implementing IPTi. The use of population Hb metrics (anemia prevalence or population mean Hb) to estimate the malaria endemicity is a valuable and easy-to-perform tool and could be added to the panel of more traditional metrics such as parasite rate.

IPTi implementation in PNG

- Despite its efficacy against *Pf* and *Pv* malaria, IPTi is not recommendable as universal policy in malaria endemic areas of PNG for the time being for the following reasons: 1) absence of efficacy on the overall morbidity and on severe illnesses. 2) Low EPI coverage in PNG. 3) Rapidly changing malaria endemicity that might jeopardize the benefits of IPTi as a control strategy. 4) Unfavourable cost-effectiveness ratio.

- If health authorities would still pursue to consider IPTi as a malaria control intervention for endemic areas of PNG, the following aspects are recommended:
    - Only a combination of two long acting drugs (SP-AQ3) is recommended for IPTi in PNG, as it is the only tested combination to be efficient on both *Pf* and *Pv* parasites.
    - EPI coverage is a fundamental aspect of IPTi effectiveness. Therefore, implementation of IPTi should be accompanied by an improvement of the overall EPI coverage. The recommended IPTi schedule is four doses at 3, 6, 9 and 12 months of age.
    - Because ITN and ACT, two efficient malaria prevention strategies, have just been recently introduced in PNG, it might be important to measure first their impact on malaria transmission prior to the implementation of IPTi. This should also help to map the regions where IPTi could be implemented as only areas with moderate to high transmission intensities can benefit from IPTi.
    - Levels of resistance of *Pf* and *Pv* against SP and AQ are high in PNG and will require to be closely monitored if IPTi would be implemented. As the first line treatment has recently changed, and SP and AQ are not any longer used as treatment, this might decrease the selection pressure of resistant parasites.
    - More generally, and because of the mentioned doubts on the real benefits of IPTi intervention in PNG, it will be essential that its effectiveness is closely monitored following its implementation. Besides malaria outcomes and overall morbidity, the impact on mortality should be especially considered as an important outcome because the trial showed a surprisingly and unexpected effect on it.

Use of RDT and AL for the management of malaria within the PNG national guidelines

- Malaria case management in small children based only on RDT results and a unified treatment for *Pf* and *Pv* using artemether/lumefantrine is safe and effective in areas endemic for different species of *Plasmodium*. This provides a confirmation that the recent decision made by PNG health authorities to introduce RDT and AL is feasible, safe and effective in routine clinics of PNG.
- RDT for malaria in PNG should include both *Pf* and non-*Pf* detection.
- Knowing the high prevalence of *Pv* infections in PNG, the use of AL alone is highly effective on initial parasitemia but does not provide any benefits for relapsing forms of hypnozoites liver-stage parasites. Strategies that could also treat these dormant forms should be considered. For example a treatment that includes a single dose of primaquine could be investigated for both its efficacy and safety in PNG.

10.2 General recommendations for IMCI guidelines

- Updating IMCI guidelines with RDT significantly improved the accuracy of malaria diagnosis and its management in small children in a region of the world highly endemic for *Pf* and *Pv*. Therefore WHO IMCI policies could be updated and complemented with RDT for malaria for all endemic areas, independently of the local epidemiology of species.
- The introduction of RDT into IMCI guidelines might lead to an overuse of antibiotics. Therefore, IMCI guidelines updated with RDT for malaria should also include clear recommendations when the RDT result is negative and appropriate training should be provided to health care providers.
- Current IMCI recommendations for the management of pneumonia are unsatisfactory leading to high rates of inaccurate diagnosis and inappropriate use of antibiotics. Therefore new strategies should be evaluated in order to be able to better identify children that need

antimicrobial therapy or more intensive support. For example, a potential strategy could be to combine more discriminative clinical criteria (increase threshold of respiratory rate and/or include fever) and objective testing for potential common aetiologies of LRTI (rapid tests) and markers of severity of LRTI such as pro-calcitonin or c-reactive protein.

- The syndromic approach of the IMCI guidelines towards diagnosis and diseases management leads to an important overlap of signs and symptoms, inaccurate diagnosis and an inappropriate use of antibiotics. It will be thus recommended to redefine the definitions of the main syndromes/diseases by introducing better clinical criteria or new diagnostic tools with increased specificities.

10.3 General recommendations for the IPTi intervention

- IPTi using SP-AQ3 is similarly effective in regions highly endemic for non-*Pf* infections compared to Africa and reduces the risk of malaria (all species) by 29%. Therefore recommendations on the use of IPTi could be generalised to all malaria endemic areas and not only Sub-Saharan Africa.
- WHO statement recommends to use in Africa IPTi-SP in regions with moderate to high malaria endemicity with “not to high” levels of resistance to SP because of its impact on malaria and severe illnesses. However, policy makers should also integrate the impact on the overall morbidity (all illness episodes, outpatient attendances) in order to provide a comprehensive picture of the overall benefits of IPTi on health.
- Among the possible reasons to explain the apparent absence of benefits on overall morbidity, in spite of a 30% reduction of malaria, is the problem of definition of “malaria disease”, as the overlap of signs and symptoms with non-malaria diseases is important. Therefore, it would be recommended to work out a better definition of “malaria disease” in order to better assess the true benefits of the intervention.

10.4 Further research areas

- Because the IPTi trial in PNG highlighted the absence of benefits on the overall morbidity and only incomplete data have been published from the African trials, it would be important to perform a pool analysis in order to investigate the impact of IPTi with SP and other drug regimens on overall morbidity and non-malaria illnesses.
- To investigate other drug combinations for IPTi with long half-life, better resistance and safety profiles and providing possible benefits on non-malaria illnesses. DHA-piperaquine might be a good candidate because of its good efficacy against *Pv.* Mefloquine could be also a good candidate for PNG, but its tolerability was poor in one IPTi study in Africa. Broad spectrum antibiotics with antimalarial activities such as azithromycine are not recommended because of the risk of promoting resistance.
- To investigate other IPT schedules than IPTi for the delivery of malaria preventive interventions (seasonal, children) in the PNG population in order to improve the efficacy on both malaria-related and -unrelated morbidities
- To identify and investigate new strategies for the diagnosis and management of respiratory infections in routine settings. These new strategies should be able to better identify 1) LRTI that need antibiotics and 2) LRTI that need more intense supportive care (hospitalisation, oxygenotherapy).
- In order to better identify new strategies towards the management of respiratory tract infections in PNG and elsewhere, it is mandatory to investigate the epidemiology of ARI and LRTI and the spectrum of causative agents.

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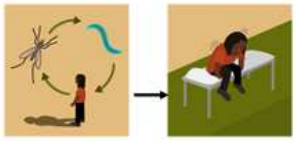
Appendix 1: Picture-based information brochure provided to parents of study participants

Bebi Klinik – IPTi Klinik

1. Wanem as tingting bilong dispela wok painimaut o stadi?

daunim namba (level) bilong sik malaria na sot blut (anemia) insait long ol liklik pikinini



2. Wanem marasin bai pikinini bilong yu i kisim?

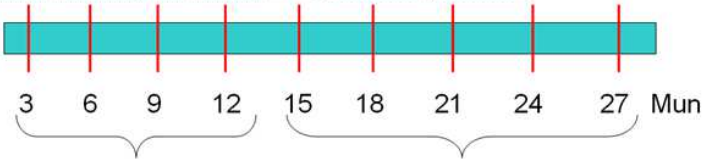
PNG Standat Marasin
Camoquine
Artesunate
Fansidar
Or Placebo

Placebo = suga

Givim Marasin long pikinini bilong yu, noken givim stadi marasin long narapela sik man/meri

3. 2000 pela pikinini bai istap insait long stadi – 4 pela yia – long ples Mugil long Madang na ples Wosera long East Sepik

4. Wanem samting bai kamap long wanwan pikinini?



5. Sapos pikinini blong yu i sik?

Kisim pikinini blong yu igo long lokol Helt Senta or Aid Post klostu long yu

- Stadi klinik tasol-

6. Wanem ol birua (risk) bilong dispela wok panimaut?

Taim mipela i kisim liklik blut long pikinini bai em pilim pen liklik o bai ikisim liklik sua tasol

Stadi marasin em i gutpela tasol sampela taim marasin iken kamapim skin sigirap na skin ret (rash)

7. Sampela Halivim long pikinini insait long stadi ?

Kamapim gutpela helt bilong pikinini

8. Olsem wanem long ol labwok?

9. Wanem pe bilong dispela wok panimaut?

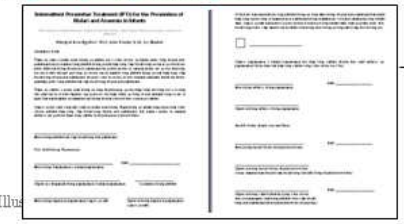
- FRI- Dispela wok panimaut bai baim ol fi bilong haus sik sapos pikinini i sik

10. Rait bilong Pikinini?

Mi laik rausim pikinini bilong mi long dispela stadi

Noken wari, em orait

11. Tok hait bilong pikinini (confidentiality)



Bai mipela i haitim ol infomesen bilong pikinini

Appendix 2: Case report forms used in the IPTi trial to record all signs, symptoms, diagnoses and treatments of illness episodes

Morbidity Form

ID Number ☐☐☐☐ **Treatment Group** ☐

1. Name of Child..... First Name ☐ Last Name ☐
2. Name of Mother/Carer..... First Name ☐ Last Name ☐
3. Name of the village..... ☐
4. Date of Birth..... Day ☐☐ Month ☐☐ Year 2 0 ☐☐
5. Date of Visit ..... Day ☐☐ Month ☐☐ Year 2 0 ☐☐
6. When illness started?... Day ☐☐ Month ☐☐ Year 2 0 ☐☐

Study Clinic HC
Mobile Team
Aid Post

7. Check for severe signs and symptoms

7.1 Unable to eat, drink or breastfeed	YES ❑ NO ❑
7.2 Vomiting everything.....	YES ❑ NO ❑
7.3 Drowsy or unconscious.....	YES ❑ NO ❑
7.4 Respiratory distress (nasal flaring or indrawing).....	YES ❑ NO ❑
7.5 Fitting (convulsions) now or in the past 24 hours.....	YES ❑ NO ❑
7.6 Inability to sit up (in a child who could usually do this) or floppy....	YES ❑ NO ❑
7.7 Neck stiffness.....	YES ❑ NO ❑
7.8 Dehydration (sunken eyes, slow skin pinch - >2s)	YES ❑ NO ❑
7.9 Pale with Heart Rate >160 or oedema.....	YES ❑ NO ❑

8. Medical history

8.1 Skin hat (history of fever)	YES	NO	**8.3 Galas (temp).....**	___ ___.___ °	
8.2 Skin yelo(pallor, anemia)	YES	NO	**8.4 Is temperature > 37.5°.....**	YES	NO

If YES to one or more of the points 8.1 to 8.4 → suspect malaria and/or anaemia and you will have to bleed the child (see section 9)

8.5 Cough.....	YES	NO	8.14 Heart Rate (/min).....	/min	
8.6 Shortness of breath.....	YES	NO	8.15 Respiratory Rate (/min).....	/min	
8.7 Running nose.....	YES	NO	8.16 Weight.....	___.___ Kg	
8.8 Diarrhoea	YES	NO	8.17 Pulse Oximetry value.....	___ %	
8.9 Abdominal pain.....	YES	NO	8.18 Crepitations	YES	NO
8.10 Vomit.....	YES	NO	If Yes.....	left	right
8.11 Ear pain.....	YES	NO	8.19 Wheezing.....	YES	NO
8.12 Skin rash.....	YES	NO	8.20 Pus out of ear.....	YES	NO
8.13 Skin spots.....	YES	NO	8.21 Others: _____	YES	NO

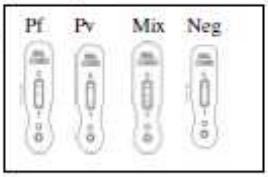
9. if you have answered YES to one or more of the question 8.1, 8.2 or 8.4, proceed to ALL blood collection bellow: (if not, don't proceed to bleeding)

9.1 Is it a regular visit?..... Y N If Yes, use bleed code of regular visit

9.2 **250µl Microtainer or filter paper** ..... Y N Bleed code X M ___ - ____

9.3 **Perform 2 blood slides**..... Y N Bleed code X M ___ - ____

9.4 **Perform RDT** *(if microscopy unavailable)* Y N If Yes, circle the result.....



9.5 **Check Hb value**..... ___ ___. ___ g/dl

9.6 Blood slide for quick read (study clinic only)..... Y N

Pf / 200 WBC		Pv / 200 WBC		Pm / 200 WBC		Po / 200 WBC		P. gam.		Negative	

- **In case of Anaemia (Hb < 8.0 g/dl) treat child according to SOP for anaemia**
- **Treat child for Malaria only if RDT or Blood Slide is positive (follow SOP)**
- **Remind parents to visit the clinic again if the child is still sick after 1-2 days**

10. Diagnosis 1. _____ 2. _____ 3. _____

11. Treatment and attitude

☐ Coartem Dose:	☐ Fefol Dose:	☐ Albendazole Dose:	☐ Amoxy Dose:	☐ Panadol Dose:	☐ Sentrine Dose:	☐ Tinidazole Dose:

☐ Other(s): _____

Child admitted at: _____ n/a

Child will be reviewed on: _____ / _____ / _____ n/a

SAE ? ☐
(Hospitalization - Life-threatening event – Death)
= RED LABEL + NOTIFY DOCTOR

11. Form completed by (initials) ..... ☐☐

.....(For IMR physician).....

Likely diagnosis: 1._____ 2. _____ 3._____

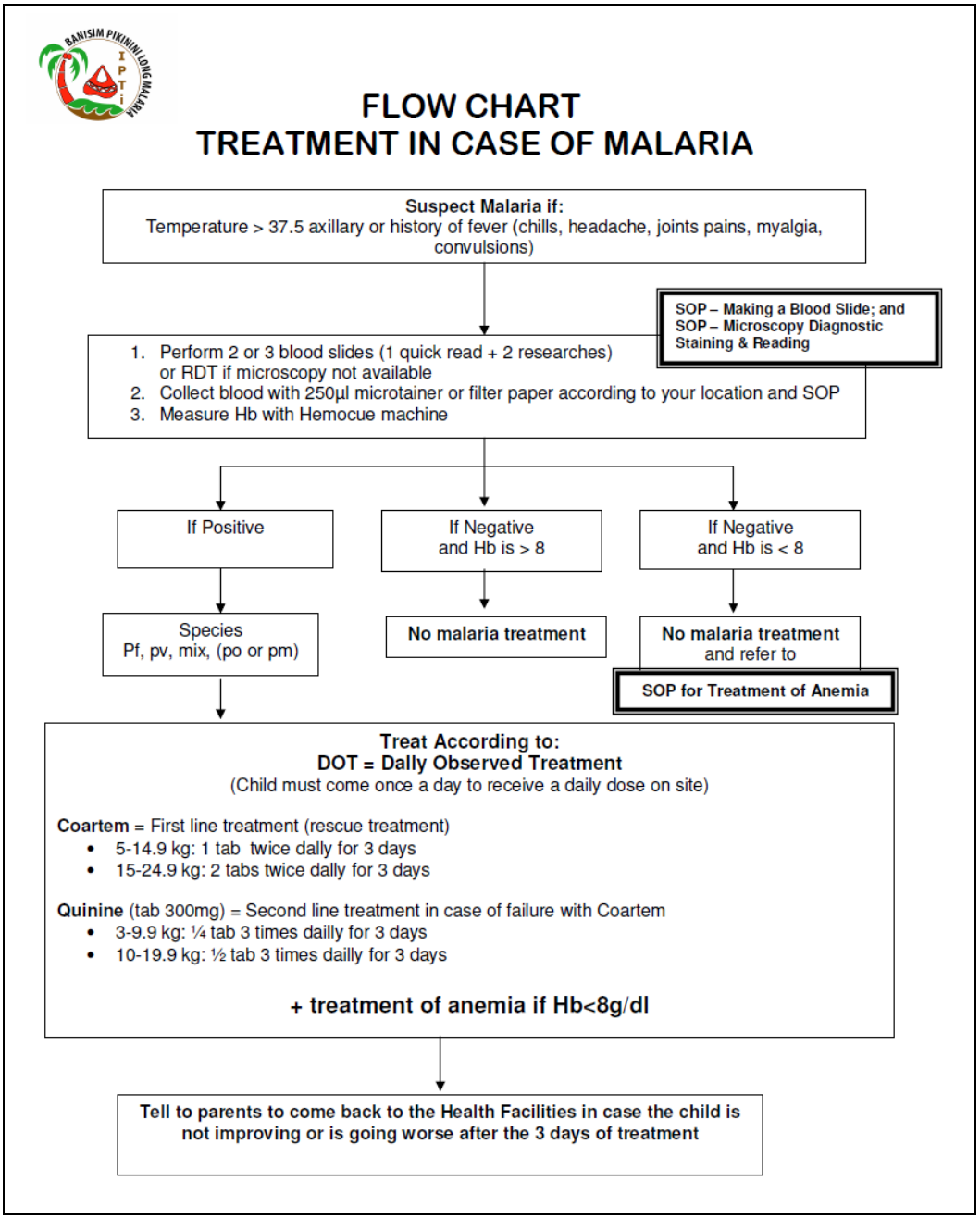
Grade of the AE: 1 2 3 SAE

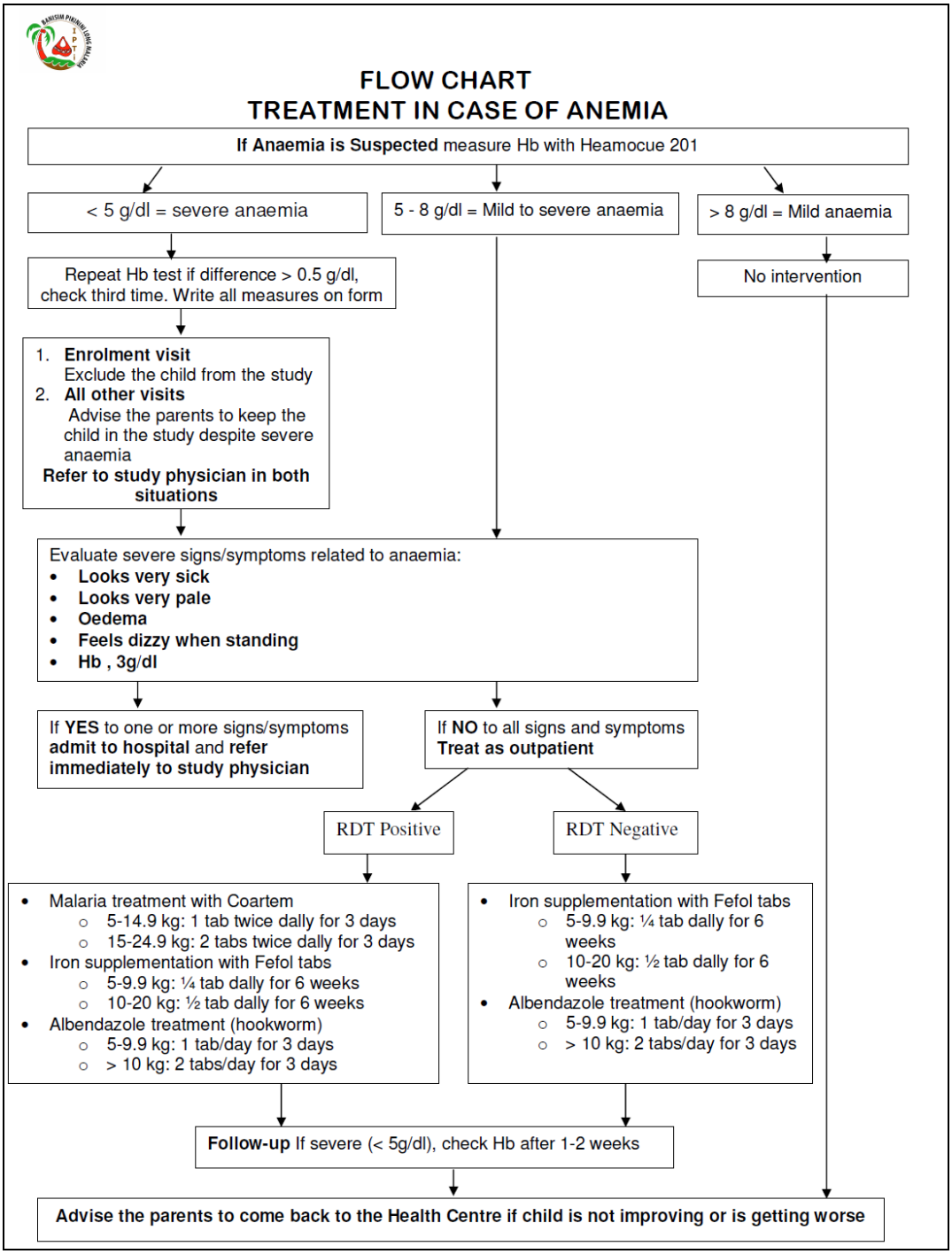
Causality of the AE: Certain / Probable / Possible / Unlikely / Unclassified

Signature: _____

If SAE:
∈ Death
∈ Hospitalization
∈ Life threatening
∈ Disability
∈ Other

Appendix 3: Treatment of malaria & anemia (IPTi trial procedures) and extracts of the PNG treatment book (IMCI) for danger signs, fever, respiratory infections, gastroenteritis and otitis




FLOW CHART
TREATMENT IN CASE OF ANEMIA
If Anaemia is Suspected measure Hb with Heamocue 201
< 5 g/dl = severe anaemia
5 - 8 g/dl = Mild to severe anaemia
> 8 g/dl = Mild anaemia
Repeat Hb test if difference > 0.5 g/dl, check third time. Write all measures on form
No intervention
1. Enrolment visit
Exclude the child from the study
2. All other visits
Advise the parents to keep the child in the study despite severe anaemia
Refer to study physician in both situations
Evaluate severe signs/symptoms related to anaemia:
Looks very sick
Looks very pale
Oedema
Feels dizzy when standing
Hb , 3g/dl
If YES to one or more signs/symptoms admit to hospital and refer immediately to study physician
If NO to all signs and symptoms Treat as outpatient
RDT Positive
RDT Negative
Malaria treatment with Coartem
5-14.9 kg: 1 tab twice dally for 3 days
15-24.9 kg: 2 tabs twice dally for 3 days
Iron supplementation with Fefol tabs
5-9.9 kg: ¼ tab dally for 6 weeks
10-20 kg: ½ tab dally for 6 weeks
Albendazole treatment (hookworm)
5-9.9 kg: 1 tab/day for 3 days
> 10 kg: 2 tabs/day for 3 days
Iron supplementation with Fefol tabs
5-9.9 kg: ¼ tab dally for 6 weeks
10-20 kg: ½ tab dally for 6 weeks
Albendazole treatment (hookworm)
5-9.9 kg: 1 tab/day for 3 days
> 10 kg: 2 tabs/day for 3 days
Follow-up If severe (< 5g/dl), check Hb after 1-2 weeks
Advise the parents to come back to the Health Centre if child is not improving or is getting worse

THE 10-STEP CHECKLIST FOR CHILDREN

Follow all the ten steps for every child

Greet the mother then ask her what is wrong with her child.

STEP (1)➔	IS THE CHILD TOO SICK? Assess for TOO SICK signs. Give immediate treatment for diagnosis found. Admit or refer the child.	p. 2
STEP (2) ➔	ASK FOR CLINIC BOOK. Check IMMUNISATION record. Give all due or overdue vaccines.	p. 50
STEP (3)➔	Use the CLINIC BOOK to check the child's GROWTH. Weigh the child and plot the weight on the weight graph. See weight chart centre pages.	
STEP (4)➔	CHECK FOR ANAEMIA. Look for pallor. If child is very pale, admit or refer urgently.	p. 6
STEP (5)➔	ASK ABOUT COUGH AND DIFFICULT BREATHING. Check for fast breathing and chest indrawing	p. 88
STEP (6)➔	ASK ABOUT DIARRHOEA. CHECK FOR DEHYDRATION. Look at the eyes and check skin elasticity	p. 34
STEP (7) ➔	ASK ABOUT FEVER. Look for signs of malaria, meningitis and other infections.	p. 45
STEP (8)➔	ASK ABOUT EAR PROBLEMS. Look for signs of otitis media and ear discharge.	p. 81
STEP (9)➔	ASK ABOUT SKIN PROBLEMS. Look for skin sores and scabies.	p. 102
STEP (10)➔	ASSESS THE CHILD'S FEEDING. Give appropriate feeding advice	p. 60

DISCUSS WITH THE MOTHER / PARENTS THE PROBLEMS THAT YOU HAVE FOUND, THE TREATMENT THAT YOU WILL GIVE AND WHAT THEY SHOULD DO. ASK ABOUT FAMILY PLANNING.

FEVER

The common causes of fever in children are:

- upper respiratory tract infection
- malaria
- otitis media
- measles
- urinary infection
- pneumonia
- meningitis
- diarrhoea
- abscess

Take a history and do a physical examination to find the cause of fever in your patient.

Collect urine for microscopy, see Paediatrics for Doctors in PNG, p. 378.

TREATMENT:

a. Treat the cause of the fever
b. **Antimalarials** (see p. 121)
c. Paracetamol to reduce fever if temperature over 38° C.

Do not give paracetamol in infants less than 3 months of age.

Over 3 months of age:

Under 10kg:	2½ml	4 times a day
10kg - 19.9kg:	5ml	4 times a day
20kg - 29.9kg:	7½ml	4 times a day
30kg or more:	10ml	4 times a day

If paracetamol is not available do NOT use aspirin in children less than 10 years old for fever reduction.

d. Give extra fluids.
e. Cool sponge if the temperature is over 38° C.

FIND THE CAUSE OF THE FEVER.
ALWAYS TREAT THIS CAUSE.

COLDS AND URTI
(Upper Respiratory Tract Infection, Simple Cough)

1. Examine the child carefully.
2. Treat as an outpatient.
3. Explain to the parents that they should come back if the child becomes short of breath.
4. Explain to the parents that the cough gets rid of rubbish from the chest and throat.

TREATMENT

a. **If no fever is present**, reassure the parents and take time to explain why the child is coughing.

b. **If fever is present**
   - **Antimalarials** (see p. 121)
   - **Paracetamol** (see p. 130)
   - if **otitis media** is present treat as on p. 81
   - if pus is present on the tonsils treat as on p. 53.

c. **Immunisation**
   Immunisation should be given if the child is due for it.
   Measles vaccine should always be given even if there is a high temperature. If the temperature is above 38°C, triple antigen and hepatitis B vaccines should be delayed until the temperature falls.

Note: Antibiotics must not be used for the treatment of colds. "Strong cough", "big cough" or "productive cough" are not indications for antibiotics unless fast breathing or another condition is also present (see p. 5).

TEACH PARENTS THE WARNING SIGNS OF PNEUMONIA: FAST BREATHING AND CHEST INDRAWING

Use a Flip Chart if you have one

PNEUMONIA OR BRONCHIOLITIS — SUMMARY

For children aged 1 month to 5 years.

	Neonates (0-1mth) with fast breathing or chest indrawing have neonatal sepsis (p. 16)			
Diagnosis		Pneumonia		
	Feature	**Mild**	**Moderate**	**Severe**
	Fast Breathing (more than 40/min)	Yes	Yes	Yes
	Chest Indrawing Present	No	Yes	Yes
	1.Pulse more than 160/min with large liver. **OR** 2.Too Sick to Suck **OR** 3.Cyanosis or Restless.	No	No	Yes
		↓	↓	↓
Management	Admit or not?	No	Yes	Yes
	Initial Antibiotic Type	Amoxycillin	Crystapen IM 6/24	Chloramphenicol IM 6/24
	Antibiotic when improved	Amoxycillin	Amoxycillin	Chloramphenicol Oral
	Total Course Duration	5 days	10 days	At least 10 days
	Other Treatment	Nil	Suction PRN	Oxygen Lanoxin oral Suction IV fluids or N/G feeds
	Malaria Treatment	A (p. 54)	A (p. 54)	B (p.55)
	Review Frequency	Daily	6 hourly	Every Hour
	Check on Review for:	Chest Indrawing	• Pulse Rate • Liver size • Cyanosis • Able to suck	• Cyanosis • Clear airway • Fever resolution

DIARRHOEA

DIARRHOEA DIAGNOSIS AND MANAGEMENT SUMMARY

↓ START HERE ↓

	A	B	C
1. LOOK AT: CONDITION..... EYES..... TEARS..... MOUTH and TONGUE..... THIRST.....	 Well, alert..... Normal..... Present..... Moist..... Drinks normally.....	 Restless, irritable..... Sunken..... Absent..... Dry..... Thirsty, drinks eagerly.....	Lethargic or Unconscious; floppy Very sunken and dry Absent Very Dry Drinks poorly or Not able to drink
2. FEEL: SKIN PINCH..... PULSE RATE..... LIMB TEMPERATURE...	 Goes back quickly.. Less than 120/min..... Warm limbs.....	 Goes back slowly (within 2 second) 120-160/min..... Warm limbs.....	 Goes back very slowly (>2 seconds) More than 160/min Cold Limbs
3. CHECK: C, then B, then A	←	←	←
4. DECIDE:	The child has **NO SIGNS OF DEHYDRATION**	If the child has <u>two or more</u> signs in col B, there is **SOME DEHYDRATION**	If the child has <u>two or more</u> signs in col C, there is **SEVERE DEHYDRATION**
5. DIAGNOSIS:	**MILD DIARRHOEA**	**MODERATE DIARRHOEA**	**SEVERE DIARRHOEA**
6. TREAT DIARRHOEA:	More fluids More often at home	Oral Rehydration Solution (ORS) under supervision	IV Half Strength Darrow's (HSD) as an in-patient
7. OTHER TREATMENT	Treat fever if present	Camoquin or Chloroquine And single dose Fansidar *Electrolyte mixture*	IM Artmether and IM Chloramphenicol *Electrolyte mixture*
8. REVIEW	Daily	Every 3-4 hours	Every hour

<u>NOTES</u>

- **Always continue breast feeding and give food to older children.**
- **Bloody diarrhea or chronic diarrhea see p. 34 and 32.**
- **Examine all children carefully for other illnesses**

OTITIS MEDIA—ACUTE

- Ear pain
  OR
- A red ear drum which is dull in appearance (poor or absent light reflex)
  OR
- Pus discharging from the ear for less than 2 weeks.

TREATMENT

a. **Amoxycillin** (250 mg tab) oral 3 times a day for 5 days.

WEIGHT	Susp dose	Tab dose
3 — 9.9kg:	5ml	½ tab
10 — 14.9kg:	7.5ml	¾ tab

WEIGHT	Tab dose
15 — 19.9kg:	1 tab
20 — 29.9kg:	1½ tab

b. If fever present:
   - Antimalarials (see **malaria** p. 54)
   - Antipyretics (see **Fever** p. 45)

c. If pus discharging:
   - **Ear cleaning with toilet tissue** (see p. 82)

d. If pus still discharging after one week of treatment:
   - **Cotrimoxazole (Septrin)** BD orally for 5 days (see p. 125).

e. If a tender swelling develops behind the ear then mastoiditis is present and the child should be referred to hospital.

CURRICULUM VITAE

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POST-GRADUATE TRAINING

2011 Post Graduate title FMH (Swiss) in general internal medicine

2010 Post-graduate exams FMH in tropical and travel medicine (speciality title)

2006 Doctorate in medicine (title: How critical is timing for the diagnosis of influenza in general practice?)

2005 Diploma in Tropical Medicine (Health Care and Management in Tropical Countries), Swiss Tropical Institute, Basel

2005 Certificate in Travel Health (International Society of Travel Medicine)

1998 Diploma in Medicine, University of Lausanne

CLINICAL EXPERIENCE

Registrar (chef de clinique)

Since 2009 Primary care outpatient Clinic, University hospital, Lausanne (50%)

2006-2008 Staff clinic for the Institute of Medical Research (part time)

2006-2008 Medical supervision (adult medicine and paediatrics) of Mugil Health Centre (part time), Papua New Guinea

2005 Part-time registrar in Travel/Tropical medicine, Centre de Vaccination et de Médecine des Voyages, University of Lausanne

Senior House Officer

2004-2005 Part-time in the HIV Clinic, University Hospital, Lausanne

2004 Oto-Rhino-Laryngology, University Hospital, Lausanne

2003 – 2004 Internal Medicine, Medical Outpatient Clinic, University of Lausanne
Tropical and Travel Medicine, Vaccinology, Travel Clinic, Medical Outpatient Clinic, University of Lausanne

House Officer

2002-2003 Internal Medicine, Regional Hospital of Morges, Switzerland (1 year)

1999-2001 General Surgery, Traumatology and Orthopedic Surgery, Regional Hospital of Riaz, Switzerland (2 years)

1999 Paediatrics, Cantonal Hospital of Fribourg (3 months)

RESEARCH EXPERIENCE

2010 Project leader for the project SPAM (Swiss Primary Health Care Active Monitoring). Long term study aiming to develop a monitoring tool centred on the quality of primary care. Performed in collaboration with the Swiss Health Observatory (Obsan).

2010 Principal investigator on a prospective research project on early diagnosis of geriatric syndromes in primary care setting (study AGE), Department of Ambulatory Care and Community Medicine, University of Lausanne, Switzerland.

2010 - 2011 Co-principle investigator for a project on electronic data collection for primary health care using portable devices (smartphone). In collaboration with HEIG-VD (haute école d'ingénieur et de gestion).

2009-2010 Coordinator for Switzerland, PHAMEU project, European monitoring project of primary health care (www.phameu.eu)

2007-08 Principal Investigator, Study on the contribution of Dengue Fever to the burden of fever diseases in Papua New Guinea and serosurvey on Measles and Rubella.

2005 - 2010 Senior coordinator and co-investigator of IPTi study (Intermittent Preventive Treatment of malaria in infant), a double blinded randomized control trial in Papua New Guinea,

2005 – 2009 Research fellow, Department of Medicine, University of Melbourne (Stephen Rogerson group)

2004-2005 Research physician in tropical/travel medicine at the CVMV (Centre de Vaccination et de Médecine des voyages), University of Lausanne under the supervision of Dr Blaise Genton

2005 Study of the impact of a brief intervention on prevention of STD in travelers and investigation of the predictors of unprotected casual sex during the travel (Principal investigator)

2004-05 Recruitment and follow-up of HIV infected patients for the HIV Swiss Cohort Study

2002 Study on the acceptability of anti-malarial chemoprophylaxis in Swiss travellers in Lausanne (Principal investigator)

2003 – 2004 Recruitment and follow-up of patients included in a clinical study assessing the feasibility and safety of web-based guidelines for the evaluation and management of fever in returning travellers and migrants **(www.fevertravel.ch).**

2001-2002 Research physician at the CVMV (Centre de vaccination et de Médecine des voyages), University of Lausanne under the supervision of Blaise Genton. Epidemiology of influenza (study on clinical predictors of influenza and prescription of anti neuraminidase agents)

PUBLICATIONS (first author)

2011 Senn N, Luang-Suarkia D, Manong D, Siba P, McBride WJ. "Contribution of Dengue fever to the burden of acute febrile illnesses in Papua New Guinea: an age-specific prospective study." ***Am J Trop Med Hyg*** **85**(1): 132-137.

2010 Senn N, Fasel E, de Valliere S, Genton B. [Gastrointestinal complaints associated to helminthes and protozoan: management by the general practitioner]. **Rev Med Suisse 2010** Dec 1;6(273):2292, 4-6, 8-301.

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2009 Senn N, Genton B. Acute hepatitis A in a young returning traveler from Kenya despite immunization before departure. ***J Travel Med*** 2009;16(1):72-3

2007 Senn N, D'Acremont V, Landry P, Genton B. Malaria chemoprophylaxis: what do the travelers choose, and how does pretravel consultation influence their final decision. ***Am J Trop Med Hyg*** 2007;77(6):1010-4

2006 Senn N, Bron L, Cavassini M. [Lymphoepithelial cysts of the parotid gland: a pathology linked to HIV infection]. ***Rev Med Suisse*** 2006;2(66):1348-50, 1352. French

2005 Senn N, Genton B. [Pre- and post-exposure rabies prophylaxis: who to vaccinate and how?]. ***Rev Med Suisse*** 2005;1(19):1280-3. French

2005 Senn N, Favrat B, D'Acremont V, Ruffieux C, Genton B. How critical is timing for the diagnosis of influenza in general practice? ***Swiss Med Wkly*** 2005;135(41-42):614-7

PUBLICATIONS (senior author)

2009 Betel Senn M, Baiwog F, Winmai J, Mueller I, Rogerson S, Senn N. nut chewing during pregnancy, Madang province, Papua New Guinea. ***Drug Alcohol Depend*** 2009;105(1-2):126-31.

OTHER PUBLICATIONS

2011 T. Cartier, N. Senn, J. Cornuz, Y. Bourgueil. The state of Primary Health care in Switzerland, the PHAMEU collaboration, NIVEL, 2011 (in press)

2010 Pell C, Straus L, Phuanukoonnon S, et al. Community response to intermittent preventive treatment of malaria in infants (IPTi) in Papua New Guinea. **Malar J** 2010;9:369.

2010 Schultz L, Wapling J, Mueller I, Senn N et al. Multilocus haplotypes reveal variable levels of diversity and population structure of Plasmodium falciparum in Papua New Guinea, a region of intense perennial transmission. **Malar J 2010**;9:336.

2010 Cornuz J, Auer R, Senn N, Guessous I, Rodondi N. [Prevention and screening: 2010 update]. **Rev Med Suisse 2010** Dec 1;6(273):2276, 8-80, 82-5.

BOARDS MEMBER

2006 - 2009 Member of the Internal Review Board (ethical committee) of the PNG Institute of Medical Research (Papua New Guinea)