

Aetiology of anaemia and public health implications in the Taabo health demographic surveillance system, south-central Côte d'Ivoire

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Summary

Background: Anaemia is a haematological condition, characterised by a decreased number of red blood cells or lower haemoglobin (Hb) concentration. Distinct cut-offs, depending on age, sex, altitude, smoking, and pregnancy status, are available from guidelines put forth by the World Health Organization (WHO), and these are widely used. Anaemia is a global public health problem with an estimated 2 billion people living with this condition worldwide. Young children and pregnant women are the population groups the most vulnerable to the consequences of anaemia, and the highest prevalence is concentrated in low- and middle-income countries. Anaemia might have subtle symptoms, including tiredness and weakness but also more severe consequences like cognition difficulties and poor pregnancy outcomes such as premature births and increased peri-natal, infant and maternal mortality. Although iron deficiency (ID) is considered as a major risk factor for anaemia, other nutritional deficiencies (e.g. folate, vitamin B12 and vitamin A), infections (e.g. *Plasmodium*, helminth and human immunodeficiency virus (HIV)) and genetic diseases (e.g., haemoglobinopathies, and glucose-6-phosphate dehydrogenase (G6PDH) deficiency) contribute to the global burden of anaemia. The choice of the diagnostic approach is a critical step in the identification and the quantification of the parameters associated with anaemia, inasmuch as several methods and biomarkers are available for the diagnosis of parasitological agents and micronutrient status with various sensitivity and specificity. Considering the broad and multifactorial aetiology of anaemia, particularly in the humid tropics, the WHO advocates integrated approaches targeting the main aetiological agents to alleviate the burden related to anaemia. Understanding the local knowledge and perceptions about blood and various anaemia-related illnesses and their relation with people's behaviour are important parameters to be accounted for. Indeed, this kind of information is crucial in the development of local sustainable intervention strategies aiming to decrease the burden of anaemia.

Goal and specific objectives: The overarching goal of this PhD thesis was to deepen our understanding of the dynamic aetiology of anaemia in the Taabo health demographic surveillance system (HDSS), south-central Côte d'Ivoire. Four specific objectives were pursued. First, to investigate the local epidemiology of anaemia, various micronutrient deficiencies, and *Plasmodium* and helminth infections in infants (6-23 months), early school-aged children (6-8 years) and young women (15-25 years). Second, to determine which socio-demographic, parasitic and nutritional variables are associated with anaemia in the three aforementioned population cohorts, including the investigation of potential interactions between multiple species parasitic infection (e.g. *Plasmodium* and hookworm), and investigate how anaemia varies over time in relation to the aforementioned parameters. Third, to assess the relation of inflammation and *Plasmodium* infection with the iron status

biomarkers soluble transferrin receptor (sTfR) and plasma ferritin (PF) in different age groups. Fourth, to study local concepts of various anaemia-related illnesses and their relationship with local health problems and people's behaviours.

Methods: The field work for this PhD was split in two parts: targeting the three first objectives, we implemented a prospective longitudinal monitoring, which started in April 2010 and included four follow-up surveys intended every 3-4 months. The end-of-study survey was conducted in June 2011. We purposely selected three settings representative of the study area, namely (i) Taabo Cité, the unique small town where there is a district hospital; (ii) Ahondo, a small village located in close proximity to the Bandama River and the Lake Taabo and (iii) Katchénou, a hamlet situated 50 km South of Taabo Cité where there was no health dispensary at the time of this study. At baseline and at the end-of-study survey, venous and finger-prick blood, stool, and urine samples were collected from the three study cohorts and subjected to standardized, quality-controlled methods to assess micronutrient and haematological status as well as parasitic infections of all participants. Finger-prick blood, stool and urine samples were also collected during the four intermediary cross-sectional surveys. At baseline, a sub-sample of venous blood samples was used to phenotype Hb. At each cross-sectional study, suspected clinical malaria, severe anaemia and helminth infections were treated according to the national guidelines of Côte d'Ivoire. For the fourth objective, a knowledge, attitudes, practices and beliefs survey was conducted in February 2012 among school-aged children and young women who had participated in the prospective longitudinal monitoring as well as newly recruited participants. This survey took place in three types of setting (town, village, and hamlet) and included quantitative data collected through a structured questionnaire and qualitative data obtained through a series of focus group discussions and semi-structured key informant interviews.

Results: The prevalence of anaemia, *P. falciparum* infection, iron, riboflavin and vitamin A deficiency are overall high in the Taabo HDSS and there are significant differences across age groups and settings. Soil-transmitted helminth and schistosome infections are focally present although with relatively low prevalence and primarily light intensities.

Whilst *P. falciparum* infection was the only parameter that showed a significant association with anaemia in infants, inflammation and cellular ID were significantly associated with higher odds of anaemia among early school-aged children. Furthermore, in this age group, *P. falciparum*-hookworm coinfection was significantly associated with lower odds of anaemia and ID as compared with *P. falciparum* infection alone. In women, we found significant positive association between cellular ID and anaemia whilst women with riboflavin deficiency or working at home had significantly lower odds of anaemia. Hb concentrations varied over

the course of the study. In infants, we observed a constant positive increase in Hb concentrations. In children and women, the dynamics of anaemia was more complex, with lower Hb concentrations found during the period of post-electoral crises (December 2010-April 2011) and significantly higher concentrations at the end-of-study survey in June 2011. In parallel, we observed a significant improvement of iron status in infants and a significant decrease of soil-transmitted helminth and schistosome infection as well as higher serum retinol concentrations in school-aged children. In women, the prevalence of schistosome infection and concentrations of α 1-acid glycoprotein were significantly lower at the end-of-study survey. These changes in haematological, nutritional and parasitic parameters were accompanied by a shift of the variables significantly associated with anaemia. In infants, *P. falciparum* infection was no more significantly associated with anaemia at the end-of-study survey. Instead, cellular ID and inflammation significantly predicted the odds of anaemia in this age group. Stunting and acute inflammation were significantly associated with anaemia in children and young women with acute inflammation had significantly higher odds of anaemia.

The results of the baseline and end-of-study surveys showed that sTfR and PF concentrations were significantly higher in infants and children with inflammation, with a similar trend observed in women and, for sTfR, this difference was independent of *P. falciparum* infection. Adjusting sTfR concentrations for inflammation in infants and school-aged children significantly decreased the prevalence of ID from 72% to 59% and from 42% to 27%, respectively. Adjusting serum PF concentration increased ID prevalence from 14% to 25% in infants but had no influence in children (ID 4%). Adjusting for *Plasmodium* infection decreased ID prevalence only in children as determined by sTfR.

Our survey about local concepts of anaemia showed that causes perceived by children and young women were based on two logical frameworks, biomedical and sociocultural, although a clear distinction was often blurred. Knowledge, beliefs and behaviours towards anaemia were relatively similar across study settings and between participants who were exposed to prior research and newly recruited participants. An important finding of this survey was the difference between the population and the health staff in understanding and preventing *djékouadjo* (local term attributed to malaria-related diseases) and nutritional issues. The population did not acknowledge *djékouadjo* as a disease exclusively transmitted by mosquitoes. Moreover, participants referred to the quantity, rather than the quality, of food when talking about nutritional issues.

Conclusions: Anaemia is an important public health problem in the Taabo HDSS in south-central Côte d'Ivoire, and this is clearly perceived by the local population. The findings of this

Summary

PhD highlight the complex aetiology of anaemia which is importantly related to infectious and nutritional issues encountered in many rural areas of tropical countries. During the years 2011-2012, many health interventions were implemented by independent national control programs and/or Taabo HDSS coordinators in the study area, which targeted malaria, helminths and, more generally, hygienic conditions and health system development. Our findings call for sustaining the achievements made and to accompany interventions by regular assessments of population health. Specific messages adapted to local concepts of anaemia and malaria might improve the acceptance and effectiveness of running programmes and future interventions. The concept of Taabo HDSS holds promise for sustained improved health, including reduced anaemia and might serve as benchmark for similar settings in sub-Saharan Africa.

Résumé

Contexte: L'anémie est un défaut hématologique caractérisé par une réduction du nombre d'érythrocytes (globules rouges) ou une diminution de la concentration en hémoglobine (Hb). Les seuils fournis par l'Organisation Mondiale de la Santé (OMS) définissant les fourchettes normales de concentration en Hb en fonction de l'âge, du sexe, de l'altitude, des habitudes tabagiques et du stade de la grossesse, servent en général de référence. L'anémie est un problème de santé publique au niveau mondial et l'OMS estime que près d'une personne sur quatre en souffre. Les enfants en bas âge et les femmes enceintes représentent les groupes les plus exposés et les plus vulnérables à l'anémie et on observe la plus haute prévalence dans les pays à bas et moyens revenus. L'anémie peut, non seulement, entraîner des symptômes subtils, comme la fatigue et la faiblesse, mais aussi des conséquences plus importantes comme des déficits cognitifs et des problèmes à la naissance, pour la mère comme pour le nouveau-né. Bien que la déficience en fer soit considérée comme un facteur de risque principal de l'anémie, d'autres déficiences nutritionnelles (folate, vitamine B12 et vitamine A), des agents infectieux (*Plasmodium ssp* (agent du paludisme), helminthes (ou vers parasites) et virus d'immunodéficience humaine (VIH)) et des maladies génétiques (problèmes de synthèse de l'Hb, déficience en glucose-6-phosphate déshydrogénase) contribuent au fardeau global de l'anémie. Le choix de la méthode de diagnostic est une étape critique dans l'identification et la quantification des variables associées à l'anémie puisque les différentes techniques et marqueurs biologiques disponibles pour les diagnostics parasitologiques et nutritionnels se caractérisent par des sensibilités et spécificités variables. Au vu de l'étiologie multifactorielle et extrêmement complexe de l'anémie, particulièrement en région tropicale, l'OMS recommande d'adopter des stratégies intégrées visant les facteurs de risque les plus importants localement, afin de diminuer le poids des conséquences de l'anémie. Les connaissances et perceptions locales du sang et de l'anémie et leur relation avec le comportement de la population font figure de paramètres indispensables à prendre en compte dans cet objectif.

But et objectifs spécifiques: Le but global de cette thèse de doctorat était d'approfondir notre compréhension de l'étiologie dynamique de l'anémie sur le site du *système de surveillance démographique sanitaire de Taabo* (SSDS de Taabo), situé en zone de rencontre sahéenne et tropicale de Côte d'Ivoire. Ce travail s'articule autour de quatre objectifs spécifiques. Le premier objectif consiste en l'étude de l'épidémiologie locale de l'anémie, des déficiences en micronutriments ainsi que des infections à *Plasmodium ssp* et aux vers parasites chez les jeunes enfants (6-23 mois), les enfants en âge d'entrer à l'école (6-8 ans) et les jeunes filles (15-25 ans). Le deuxième objectif est l'identification des variables sociodémographiques, parasitologiques et nutritionnels associées à l'anémie dans

ces trois groupes d'âges, incluant les interactions potentielles entre différents groupes de parasites (par ex. *P. falciparum* et ankylostomes), ainsi que l'évaluation de l'évolution de l'anémie à travers le temps en relation avec les facteurs précités. Évaluer l'effet de l'inflammation ou de l'infection à *P. falciparum* sur les marqueurs biologiques du statut en fer que sont le récepteur soluble à la transferrine (sTfR) et la ferritine plasmatique (PF) dans différents groupes démographiques constitue le troisième objectif. Le dernier objectif se réfère à l'étude des concepts locaux de l'anémie et leur relation avec le comportement de la population et les problèmes de santé locaux.

Méthodes: Le travail de terrain a été réalisé en deux étapes. Les trois premiers objectifs ont fait l'objet d'une étude prospective longitudinale de 14 mois, qui a débuté au mois d'avril 2010 et comprenait une étude de suivi tous les 3-4 mois. L'enquête finale s'est déroulée en juin 2011. Nous avons sélectionné trois sites d'études représentatifs de l'ensemble de la zone du SSDS de Taabo, soit (i) Taabo Cité, l'unique petite ville du SSDS où se trouve l'hôpital général de la sous-préfecture de Taabo, (ii) Ahondo, un village situé à proximité du Bandama et du Lac de Taabo où l'on trouve un centre de santé rural tenu par un infirmier et (iii) Katchénou, un hameau reconnu plus tard comme village de la sous-préfecture, qui ne disposait pas, au moment de l'étude, de son propre centre de santé. Lors de l'étude de base et de l'étude finale, un échantillon de sang capillaire, un échantillon de sang veineux, un échantillon de selles et un échantillon d'urine ont été collectés auprès de chaque participant. Pour les quatre études intermédiaires, les mêmes échantillons ont été collectés, hormis le sang veineux. Lors de chaque enquête, les participants présentant un paludisme clinique, une anémie sévère ou une infection par des vers parasites ont reçu un traitement spécifique à leur diagnostic. Le quatrième objectif a fait l'objet d'une étude transversale, en février 2012, des connaissances, attitudes, pratiques et croyances relatives au sang et à l'anémie chez les enfants et les jeunes femmes ayant participé à l'étude longitudinale ainsi que chez d'autres individus n'ayant pas été exposés à ce travail de recherche. Cette dernière étude a pris place dans les trois types de localités caractéristiques du SSDS (petite ville, village et hameau) et s'est déclinée en une collecte de données quantitatives, à l'aide d'un questionnaire structuré, et qualitatives, obtenues dans le cadre de discussions focalisées de groupe et d'interviews semi-structurées d'informateurs clés.

Résultats : L'anémie, les infections à *P. falciparum* et les déficiences en fer, en riboflavine et en vitamine A présentent des prévalences globalement hautes dans la zone d'étude, avec néanmoins des différences significatives entre les différents groupes d'âge et les différents types de localités. Les infections aux ankylostomes et aux schistosomes montrent une répartition focale dont la prévalence et les intensités sont relativement peu élevées.

Nos résultats indiquent qu'au commencement de l'étude, l'infection à *P. falciparum* était le seul paramètre associé de manière significative à l'anémie chez les jeunes enfants, alors que les enfants d'âge scolaire présentant une inflammation ou une déficience en fer avaient une probabilité significativement plus élevée d'être anémiés. De plus, nos résultats indiquent que les enfants d'âge scolaire atteints d'une coinfection à *P. falciparum* et aux ankylostomes présentaient un moindre risque d'anémie par rapport aux enfants uniquement infesté par *P. falciparum*. Chez les femmes, la déficience en fer était significativement associée à la probabilité d'être anémiée alors que l'association entre l'anémie et une déficience en riboflavine ou une activité exclusivement domestique était significativement négative. On observe une variation des concentrations en Hb durant l'étude, dans toutes les classes d'âges. Cependant, alors qu'on observe une augmentation plus ou moins constante de la concentration en Hb chez les jeunes enfants, la dynamique de l'anémie se révèle plus complexe chez les enfants d'âge scolaire et les jeunes femmes. En effet, dans ces deux groupes d'âges, on note une diminution de la concentration en Hb durant la période de crise postélectorale qu'a traversée la Côte d'Ivoire entre décembre 2010 et avril 2011, puis une augmentation significative du taux d'Hb lors de l'étude finale, en juin 2011. En parallèle, nous assistons à une amélioration significative du statut en fer chez les jeunes enfants ainsi qu'à une diminution significative des infestations aux vers parasites (ankylostomes et schistosomes) et une augmentation de la concentration en rétinol plasmatique chez les enfants d'âge scolaire. Chez les femmes, les infestations aux schistosomes et la concentration médiane en alpha-1-glycoprotéine acide (marqueur de l'inflammation) étaient significativement moins importantes à la fin de l'étude. Ces changements s'accompagnent d'une modification des variables associées à l'anémie dans les trois classes d'âges. En effet, à la fin de l'étude, la déficience en fer et l'inflammation sont associées à une probabilité significativement plus élevée d'anémie chez les jeunes enfants. Chez les enfants d'âge scolaire, l'anémie est associée de manière significative à la malnutrition et à l'inflammation aiguë, cette dernière variable étant par ailleurs aussi associée à l'anémie chez les jeunes femmes.

Les résultats de l'étude de base et de l'étude finale montrent que les enfants atteints d'inflammation ou d'infection à *P. falciparum* présentent des concentrations significativement plus élevées en sTfR et en PF que les enfants sans inflammation ou sans infection à *P. falciparum*. On observe une tendance similaire, bien que non-significative, chez les jeunes femmes. Pour le sTfR, l'effet de l'inflammation était indépendant de l'infection à *P. falciparum*. Chez les jeunes enfants et les enfants d'âges scolaires, la correction des concentrations de sTfR pour l'effet de l'inflammation diminue significativement la prévalence de la déficience en fer, de 72% à 59% et de 42% à 27%, respectivement. La correction de

PF augmente la prévalence de la déficience en fer de 14% à 25% chez les jeunes enfants, mais n'a pas d'effet chez les enfants d'âge scolaire (4%). L'ajustement pour l'effet de *P. falciparum* abaisse de manière significative la prévalence de la déficience en fer chez les enfants d'âge scolaire uniquement.

Notre étude sur les concepts locaux de l'anémie a montré que les causes perçues par les enfants et les jeunes femmes correspondent à deux bases logiques, l'une biomédicale, l'autre socioculturelle, bien qu'une distinction entre ces deux logiques ne soit pas toujours évidente. Les connaissances, les croyances et les comportements par rapport à l'anémie étaient relativement similaires entre les différents types de localité et entre les individus ayant participé à notre étude longitudinale et ceux n'ayant pas été exposés à ce travail de recherche. Une conclusion importante de cette enquête est la différence importante qui existe entre la population et la structure sanitaire quant à la description et à la prévention de *djékouadjo* (terme local attribué au paludisme) et des problèmes nutritionnels. En effet, la population ne reconnaît pas *djékouadjo* comme maladie transmise uniquement par le moustique. D'autre part, la population se réfère à la quantité, plutôt qu'à la qualité de la nourriture, en parlant des problèmes de nutrition.

Conclusion: L'anémie est un problème de santé publique important dans le SSDS de Taabo, en zone sud centrale de Côte d'Ivoire, bien connu de la population locale. Les résultats de cette thèse soulignent l'étiologie complexe de l'anémie, intimement liée à des problèmes nutritionnels et infectieux rencontrés dans nombre de zones rurales tropicales. Pendant et après notre étude, durant les années 2010 et 2012, plusieurs interventions sanitaires ont été déployées dans la zone du SSDS de Taabo. Ces interventions, coordonnées par des programmes nationaux indépendants et/ou l'équipe coordinatrice du SSDS de Taabo, ciblaient le paludisme, les vers parasites ou, de manière plus générale, les conditions hygiéniques et le développement du système de santé. Nos résultats soulignent l'importance de continuer ces interventions, tout en évaluant régulièrement l'état de santé de la population. Des messages spécifiques adaptés aux concepts locaux de l'anémie et du paludisme pourraient contribuer à améliorer l'acceptation et l'efficacité des programmes en place et des interventions futures. Le concept du SSDS de Taabo se révèle prometteur pour une amélioration durable non seulement des taux d'Hb mais aussi de la situation sanitaire globale et pourrait faire office de référence pour d'autres sites d'Afrique sub-saharienne.

Zusammenfassung

Hintergrund: Anämie (umgangssprachlich auch Blutarmut genannt) ist eine Bluterkrankung, welche durch eine reduzierte Erythrozytenanzahl (Rote Blutkörperchen) oder eine verminderte Hämoglobinkonzentration gekennzeichnet werden kann. In den Richtlinien der Weltgesundheitsorganisation (WHO) sind klare Grenzwerte – abhängig von Alter, Geschlecht, Höhenlage, Rauchen und Schwangerschaft – definiert, die weitgehend verwendet werden. Anämie ist ein weltweit vorkommendes Gesundheitsproblem, das geschätzte 2 Milliarden Menschen betrifft. Zu den gefährdetsten Bevölkerungsgruppen der Folgen einer Anämie gehören jüngere Kinder und schwangere Frauen. Die Prävalenz ist am höchsten in Ländern mit tiefen und mittleren Einkommen (*low- and middle income countries*). Anämie kann sich über leichte Symptome wie Müdigkeit und Kraftlosigkeit bemerkbar machen, kann aber auch schwere Folgen wie Wahrnehmungs- oder Persönlichkeitsstörungen und verminderte Schwangerschaftsergebnisse mit sich bringen. Obwohl der Eisenmangel als Hauptrisikofaktor einer Anämie gilt, tragen Defizite über die Ernährung (wie z.B. Folsäure-, Vitamin B12- und Vitamin A-Mangel), Infektionen (z.B. *Plasmodium*-, Helmintheninfektionen und Ansteckungen mit dem Humanen Immundefizienz Virus (HIV)) und Erbkrankheiten (wie z.B. Hämoglobinopathien und Glucose-6-Phosphat-Dehydrogenase-Mangel) zur globalen Bürde der Anämie bei. Diagnostische Mittel zur Identifizierung und Quantifizierung von Anämie assoziierten Parametern müssen sorgfältig gewählt werden, da verschiedene Methoden und Biomarker mit unterschiedlichen Sensitivitäten und Spezifitäten für die Diagnose von parasitologischen Befunden, aber auch zur Erhebung des Mikronährstoffhaushalts zur Verfügung stehen. Unter Berücksichtigung der breiten und multifaktoriellen Ätiologie der Anämie vertritt die WHO speziell für das Afrika südlich der Sahara integrierte Herangehensweisen, welche auf die wichtigsten Ursachen der Anämie abzielen um die der Anämie unterzuordnende Bürde zu lindern. In diesem Zusammenhang werden das vorhandene lokale Wissen, die Wahrnehmungen über Blut und Anämie zugehöriger Krankheiten und deren Verbindung zum persönlichen Umgang damit als wichtige Parameter erwähnt. Diese Art von Informationen ist unabdingbar für die Entwicklung einer lokalen, nachhaltigen Interventionsstrategie, mit dem Ziel der Reduzierung der durch die Anämie verursachten Bürde.

Ziel: Das allumfassende Ziel dieser Dissertation (PhD) war das Wissen über die dynamischen ätiologischen Prozesse der Anämie innerhalb des Taabo Demographie- und Gesundheitsüberwachungssystems (Taabo HDSS) im südzentral gelegenen Teil der Elfenbeinküste zu vertiefen. Um das allumfassende Ziel zu erreichen wurden vier spezifische Anliegen verfolgt: Erstens, die Erörterung der lokalen Epidemiologie der Anämie, anhand von unterschiedlichen Mikronährstoffdefiziten, *Plasmodium*- und Helmintheninfektionen von

Kleinkindern (6-23 Monate alt), Kindern im frühen Schulalter (6-8 Jahre alt) und jungen Frauen (15-25 Jahre alt); Zweitens, die Bestimmung der soziodemographischen, parasitischen und Ernährungs-Parameter, welche in unseren drei zuvor erwähnten Bevölkerungskohorten mit der Anämie einhergehen, und folglich zu untersuchen, wie sich die Anämie in Bezug auf die oben genannten Parameter mit der Zeit verändert unter Einbezug der Untersuchung potentieller Interaktionen zwischen Co-Infektionen unterschiedlicher Arten (z.B. gleichzeitige *Plasmodium*- und Hakenwurminfektion); Drittens, die Abschätzung der Beziehungen von Entzündungen und *Plasmodium*-Infektionen zu den beiden Eisenhaushalt-Biomarkern löslicher Transferrin-Rezeptor (*soluble transferrin receptor*, sTfR) und Plasma Ferritin (PF) für die unterschiedlichen Altersgruppen; und Viertens, die Untersuchung lokaler Vorstellungen zu den diversen Anämie zugehörigen Krankheiten und ihren Zusammenhang zu individuellem Verhalten und lokalen Gesundheitsproblemen.

Methoden: Die Feldarbeit dieser Dissertation wurde in zwei Teile aufgeteilt: um den ersten drei Anliegen gerecht zu werden, wurde ein prospektives longitudinales Monitoring mit Start im April 2010 und vier Nachuntersuchungen idealerweise im Abstand von drei bis vier Monaten durchgeführt. Das longitudinale Monitoring endete im Juni 2011. Drei Altersgruppen wurden in das Monitoring einbezogen, (i) Kleinkinder (im April 2010 6-23 Monate alt), (ii) Kinder im frühen Schulalter (6-7 Jahre alt) und (iii) junge Frauen (15-25 Jahre alt). Drei das Studiengebiet repräsentierende Settings wurden ausgewählt, namentlich (i) Taabo Cité, die einzige Kleinstadt mit einem Bezirksspital; (ii) Ahondo, ein Dorf in näherer Umgebung des Bandama Flusses und des Taabo Stausees und (iii) Katchénou, ein 50 km südlich von Taabo Cité gelegenes Dörfchen, das zur Zeit dieser Studie keine Arzneiausgabestelle hatte. Zu Beginn der Studie (*Baseline*) und zum Abschluss der Studie (*End-of-study*) wurden venöses Blut und Fingerstichblut, Stuhl- und Urinproben aller Teilnehmenden der drei Bevölkerungskohorten gesammelt und mittels standardisierten, qualitätskontrollierten Methoden sowohl auf Mikronährstoffe, als auch auf parasitische Infektionen untersucht und jeweils der hämatologische Zustand des Blutes determiniert. Fingerstichblut, Stuhl- und Urinproben wurden zudem an den vier dazwischenliegenden Nachuntersuchungen genommen. Zu Beginn der Studie wurde eine Teilprobe des venösen Blutes für die Phänotypisierung des Hämoglobins verwendet. Zum Zeitpunkt jeder Nachuntersuchung wurden mutmassliche klinische Malariafälle, schwere Anämien und Helmintheninfektionen den nationalen Richtlinien der Elfenbeinküste entsprechend behandelt. Für das vierte Anliegen wurde im Februar 2012 eine Umfrage zu Wissen, Haltungen, Handhabungen und Glauben sowohl mit Kindern im Schulalter und jungen Frauen aus dem prospektiven longitudinalen Monitoring als auch mit neuen Teilnehmenden durchgeführt. Die Umfrage

wurde in drei verschiedenen Settings (Kleinstadt, Dorf, Dörfchen) durchgeführt und beinhaltete einerseits eine quantitative Sammlung von Daten mittels eines gegliederten Fragebogens und andererseits die qualitative Erlangung von Informationen über Fokusgruppendifkussionen und halbgegliederte Interviews mit Schlüsselinformanten.

Resultate: Die Prävalenzen von Anämie, *Plasmodium*-Infektionen, Eisen-, Riboflavin- und Vitamin A-Mängel sind innerhalb des Taabo HDSS hoch und weisen signifikante Unterschiede innerhalb der Altersgruppen und Settings auf. Wenngleich bodenübertragene Helminthen- und Schistosomeninfektionen lokal vorkommen sind deren Prävalenzen und Intensitäten eher niedrig.

Während *Plasmodium falciparum* der einzige Parameter war, der signifikant mit einer Anämie assoziiert bei Kleinkindern vorlag, fanden wir heraus, dass Entzündungen und zellulärer Eisenmangel signifikant mit den Odds von Anämie bei Kindern im Schulalter einhergehen. Des Weiteren sind *Plasmodium*-Hakenwurm Co-Infektionen in der selben Gruppe mit signifikant tieferen Odds von Anämie und Eisenmangel assoziiert im Vergleich zu *Plasmodium*-Infektionen allein. Bei Frauen fanden wir eine positive signifikante Assoziation zwischen zellulärem Eisenmangel und Anämie während Frauen mit Riboflavinmangel oder Frauen, die angaben, zu Hause zu arbeiten, signifikant tiefere Odds für Anämie aufwiesen. Die Hämoglobinkonzentrationen variierten über die ganze Dauer der Studie hinweg. Bei den Kleinkindern konnten wir einen konstanten Anstieg der Hämoglobinkonzentration beobachten. Bei den Kindern im Schulalter und den jungen Frauen hingegen war die Dynamik der Anämie komplexer, mit tieferen Hämoglobinkonzentrationen während der Zeitspanne nach der Wahlkrise (Von Dezember 2010 bis April 2011) und signifikant höheren Konzentrationen bei der Untersuchung am Abschluss der Studie im Juni 2011. Parallel dazu konnten wir eine signifikante Erhöhung des Eisenhaushalts in Kleinkindern und eine signifikante Abnahme von bodenübertragenen Helminthen- und Schistosomeninfektionen ebenso wie höhere Serum Retinol Konzentrationen in Kindern im Schulalter beobachten. Die Prävalenzen von Schistosomeninfektionen und Konzentrationen von alpha1-Säure-Glycoproteinen bei den Frauen waren signifikant tiefer zum Abschluss der Studie. Diese Veränderungen in hämatologischen, parasitischen und Ernährungs-Parametern wurden begleitet von einer Verlagerung der mit Anämie signifikant assoziierten Variablen. Bei den Kleinkindern waren *Plasmodium*-Infektionen zum Abschluss der Studie hin nicht mehr signifikant mit Anämie assoziiert. Statt dessen bestimmten in dieser Altersgruppe zellulärer Eisenmangel und Entzündungen die Odds für Anämie. Vermindertes Wachstum und akute Entzündungen waren bei den Kindern signifikant mit Anämie assoziiert und junge Frauen, die eine akute Entzündung aufwiesen, hatten signifikant höhere Odds für Anämie.

Die Resultate der Baseline und End-of-study Untersuchungen zeigten, dass die sTfR und PF Konzentrationen bei den Kleinkindern und den Kindern mit Entzündungen signifikant höher waren. Ein ähnlicher Trend wurde auch bei den Frauen beobachtet. Der beobachtete Unterschied von sTfR war unabhängig vom *Plasmodium*-Infektionsstatus. Eine Anpassung der sTfR Konzentrationen an Entzündungswerte verringerte die Prävalenz von Eisenmangel in Kleinkindern von 72% auf 59% und Kindern im Schulalter von 42% auf 27%. Eine Angleichung der PF Konzentration führte in Kleinkindern zu einem Anstieg der Eisenmangel Prävalenz von 14% auf 25% hatte aber keine Auswirkung bei Kindern (ID 4%). Eine Anpassung von sTfR Werten an *Plasmodium* Infektionen verringerte die Eisenmangel Prävalenz nur in Kindern.

Unsere Studie zu den lokalen Vorstellungen der Anämie zeigte, dass Gründe, die von Kindern und jungen Frauen wahrgenommen wurden auf zwei folgerichtigen biomedizinischen und soziokulturellen Rahmenbedingungen beruhten, obwohl eine klare Unterscheidung oft nicht möglich war. Wissen, Glaube und Verhalten in Bezug auf Anämie waren in allen Studien-Settings und bei den Teilnehmenden, die bereits an früheren Forschungs-Studien teilgenommen hatten oder neu hinzugekommen waren, ähnlich. Ein wichtiges Ergebnis der vorliegenden Studie war, dass es einen Unterschied im Verständnis und der Vorbeugung von *djékouadjo* (örtlicher Ausdruck für Erkrankungen im Zusammenhang mit Malaria) gab. Die Bevölkerung nahm *djékouadjo* nicht als Erkrankung war, die ausschliesslich von Moskitos übertragen wird. Ferner kam hinzu, dass die Teilnehmenden auf die Quantität statt auf die Qualität von Nahrung verwiesen, wenn sie über Ernährung sprachen.

Schlussfolgerung: Anämie ist ein wichtiges öffentliches Gesundheitsproblem innerhalb des Taabo HDSS in der südzentralen Elfenbeinküste und wird deutlich als solches von der lokalen Bevölkerung anerkannt. Die Ergebnisse dieser Dissertation heben die komplexe Ätiologie der Anämie hervor, welche die Verstrickung mit Infektions- und Ernährungsproblemen, vorgefunden in zahlreichen ruralen Gegenden tropischer Länder, verdeutlicht. Während der Jahre 2010-2012 wurden mehrere Gesundheitsinterventionen von unabhängigen nationalen Kontrollprogrammen und/oder der Taabo HDSS Koordination in der Studienregion implementiert, welche die Malaria, Helminthen und - ganz generell - hygienischen Bedingungen und die Ausarbeitung des Gesundheitssystems ins Auge fassten. Unsere Resultate verdeutlichen die Notwendigkeit für einen dauerhaften Unterhalt dieser Interventionen, die durch regelmässige Gesundheitskontrollen in der Bevölkerung unterstützt werden. Eigens für die lokalen Konzepte der Anämie und Malaria angepasste Botschaften könnten die Akzeptanz und die Effizienz zukünftiger Programme und Interventionen erhöhen. Das Konzept des Taabo HDSS stellt einen vielversprechenden Rahmen für eine verbesserte

Gesundheitssituation - inklusive reduzierter Anämie - dar, welches als Massstab auf ähnliche Settings in anderen Ländern des subsaharischen Afrikas angewendet werden könnte.

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List of abbreviations

¹H-NMR, proton nuclear magnetic resonance
ACD, anaemia of chronic diseases
ACTs, artemisinin-based combination therapies
AGP, alpha 1-acid glycoprotein
AIC, Akaike's information criterion
AIDS, acquired immunodeficiency syndrome
BM, bone marrow
BMI, body mass index
CCA, circulating cathodic antigen
CF, correction factor
CI, confidence interval
CRP, C-reactive protein
CSRS, Centre Suisse de Recherches Scientifiques en Côte d'Ivoire
DALYs, disability-adjusted life years
EGR, erythrocyte glutathione reductase
EGRAC, erythrocyte glutathione reductase activity coefficient
ELISA, enzyme-linked immunosorbant assay
EPG, eggs/g of stool
FAO, Food and Agriculture Organization of the United Nations
FGD, focus group discussion
G6PDH, glucose-6-phosphate dehydrogenase
HAZ, height-for-age Z-score
Hb, haemoglobin
HDSS, health demographic surveillance system
HIF-1, hypoxia-inducible factor-1
HIV, human immunodeficiency virus
HPLC, high-pressure liquid chromatography
ID, iron deficiency
IDA, iron deficiency anaemia
INDEPTH, international network for the demographic evaluation of populations and their health in developing countries
IPT, intermittent preventive treatment
IRA, indoor residual spraying
KAPB, knowledge, attitudes, practices and beliefs
LLIN, long-lasting insecticidal net

MCH, mean corpuscular haemoglobin

MCV, mean corpuscular volume

MS, mass spectrometry

N/A, not applicable

NGO, non-governmental organisation

Pf, *Plasmodium falciparum*

PF, plasma ferritin

PCR, polymerase chain reaction

PhD, Doctor of Philosophy

RA, retinoic acid

RDT, rapid diagnostic test

RBC, red blood cell

RBP, retinol-binding protein

SD, standard deviation

SE, standard error

SNSF, Swiss National Science Foundation

Swiss TPH, Swiss Tropical and Public Health Institute

sTfR, soluble transferrin receptor

ThX, T helper lymphocytes (Th1 activates the cellular immune response; Th2 activates the humoral immune response)

VAD, vitamin A deficiency

WAZ, weight-for-age Z-score

WHO, World Health Organization

ZPP, zinc protoporphyrin

1. Introduction

This chapter provides a succinct overview of the definition of anaemia (section 1.1), its public health burden (section 1.2), multifactorial aetiology (section 1.3) and the consequences of anaemia (section 1.4). In section 1.5, I introduce different methods for the diagnosis of anaemia, including biomarkers used in the assessment of its aetiological agents. Finally, in section 1.6, measures for the prevention and control of anaemia are summarised, placing particular emphasis on the situation in Africa, south of the Sahara.

1.1 *Definition of anaemia*

Anaemia stems from ancient Greek “ἀναιμία”, which means “without blood”. The biomedical definition of anaemia is a reduction in the number of red blood cells (RBC) or the haemoglobin (Hb) content of blood, or a decreased ability of Hb to bind oxygen (Schnall, 2000). In clinical terms, anaemia is considered as an Hb concentration that is insufficient to meet the oxygen needs of the tissues and distinct cut-offs are available in guidelines put forward by the World Health Organization (WHO) for different age groups and males and females.

1.2 *Burden of anaemia*

Anaemia is a global public health problem which affects all population groups in low-, middle- and high-income countries, with an estimated 2 billion people living with this condition (**Figure 1.1**) (WHO/UNICEF/UNU, 2001). Pregnant women and young children are the population groups that are at highest risk of anaemia, particularly in sub-Saharan Africa, South America and Southeast Asia (McLean et al., 2009). The global burden of anaemia, in terms of disability-adjusted life years (DALYs) is hard to quantify, as anaemia can result from various diseases and other conditions of ill-health. However, iron deficiency anaemia (IDA) has been estimated to cause 16 million DALYs worldwide and between 190,000 and 974,000 children under 5 years of age die each year from malaria-associated anaemia (Murphy and Breman, 2001; WHO, 2008).

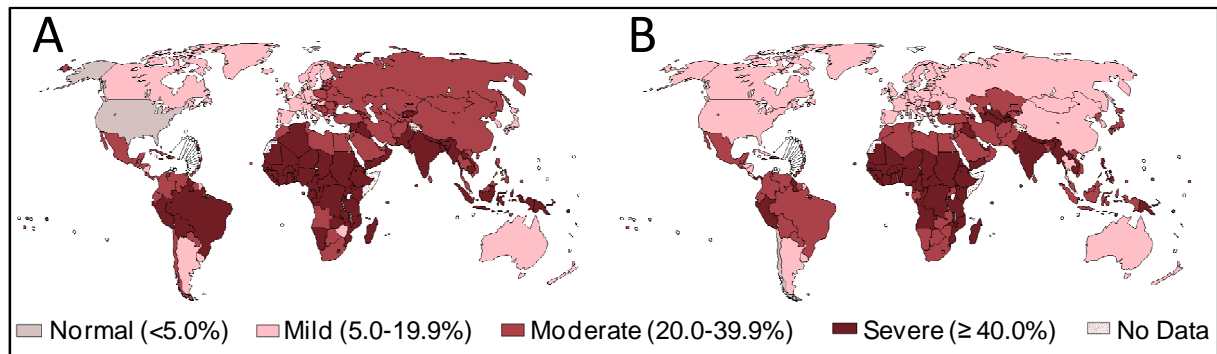


Figure 1.1: Worldwide prevalence of anaemia.

Prevalence among (A) preschool-aged children and (B) non-pregnant women (source: McLean et al., 2009)

1.3 Aetiology of anaemia

The common belief that iron deficiency (ID) is the main cause of anaemia worldwide mainly comes from estimates which used Hb as a proxy to estimate the prevalence of ID (Stoltzfus 2001). Nevertheless, as shown in **Figure 1.2**, anaemia is multifactorial. Indeed, anaemia can result from other nutritional deficiencies such as folate, vitamin B12 or vitamin A (Suharno et al., 1993; Savage et al., 1994; Stabler and Allen, 2004), or from parasitic diseases such as malaria (Menendez et al., 2000) and helminthiases (Stephenson et al., 1985; Brooker et al., 2004; Friedman et al., 2005), as a consequence from chronic inflammatory diseases (Yip and Dallman, 1988) or from genetic disorders such as haemoglobinopathies (Stuart and Nagel, 2004; Rund and Rachmilewitz, 2005), or glucose-6-phosphate dehydrogenase deficiency (Cappellini and Fiorelli, 2008). Anaemia can be the consequence of a decreased production of RBC, an increased destruction of RBC and/or direct blood loss.

1.3.1 Micronutrients deficiency

Malnutrition is considered as a leading direct or indirect cause of death for children below the age of 5 years in developing countries (Black et al., 2003). In the initial estimation of the global burden disease, published in 1997, Murray and Lopez estimated that “15.9% of DALYs worldwide are attributable to childhood malnutrition” (Murray and Lopez, 1997). Furthermore, it has been shown that poor nutrition and micronutrient deficiencies may exacerbate the severity of infectious diseases (Scrimshaw and SanGiovanni, 1997).

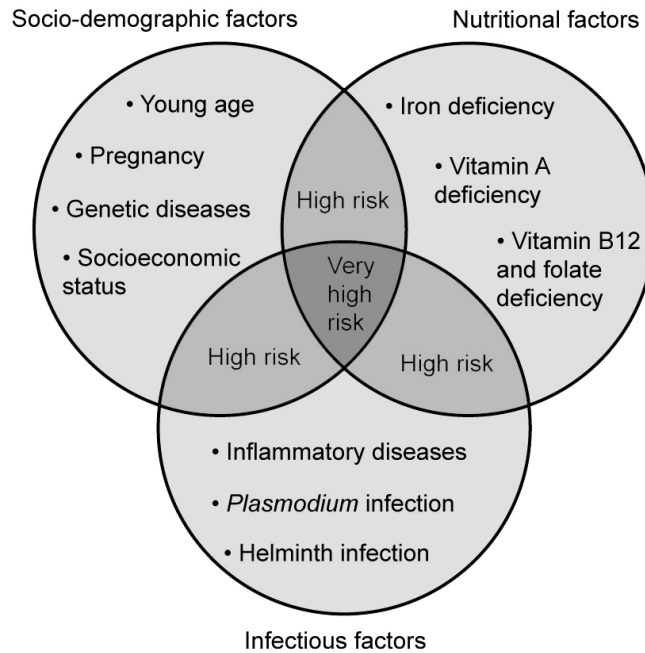


Figure 1.2: Potential risk factors for anaemia.

In tropical settings, the aetiology of anaemia is multifactorial. This Venn's diagram depicts a hypothetical risk ranking of anaemia in relation to socio-demographic, nutritional and infectious factors. Of note, the interplay between different agents may be much more complex.

1.3.1.1 Iron deficiency

ID is the most common and widespread nutritional disorder in the world and IDA is considered as an important contributing factor to the global burden of disease (Murray and Lopez, 1997). In Africa, previous estimates suggest that half of the children and women of childbearing age, particularly those living in deprived settings, suffer from ID. However this estimate may not be reliable because anaemia has widely been used as a proxy for ID (WHO/UNICEF/UNU, 2001). Data for IDA in Côte d'Ivoire vary depending on region, age, sex and the choice of biomarkers (Staubli-Asobayire et al., 2001; Wegmüller et al., 2006, Rohner et al., 2010).

The early stage of ID can be recognized by abnormalities in serum proteins, whereas the more advanced stage consists in IDA (Handelman and Levin, 2008). Because anaemia is the most common indicator for ID, the terms anaemia, ID and IDA are sometimes used interchangeably. However there are forms of ID where anaemia is absent and anaemia can appear independently of ID, especially in areas that are endemic for malaria and other parasitic diseases (Staubli-Asobayire et al., 2001; Crawley, 2004). Thus, ID and anaemia are not the exact same issue and hence they should be considered independently.

Iron homeostasis is finally regulated and iron overload can also be detrimental for health (Tapiero et al., 2001; Weiss, 2005). In malaria-endemic regions, complex interactions exist between the host's iron status, the malaria parasite and the immune system (Prentice et al. 2007). A survey conducted in Pemba, Tanzania, highlighted that supplementation with iron and folic acid should not be administered to iron-replete children (Sazawal et al., 2006). This finding guided new recommendations by the WHO for the control of ID in children living in malaria-endemic countries, namely that universal iron supplementation should not be implemented without the screening of individuals for ID (WHO, 2007). However, these guidelines are still being debated, partly because the mechanisms involved in adverse events of iron supplementation are yet to be fully elucidated (Verhoef and Veenemans, 2009; Brittenham, 2011).

1.3.1.2 Vitamin A deficiency

There is a growing body of evidence that vitamin A also plays an important role in iron homeostasis. Vitamin A is needed for erythropoiesis and for the mobilization of iron from the spleen and liver stores (Roodenburg et al., 2000). Retinoic acid (RA), the oxidized form of vitamin A, up-regulates the production of erythropoietin in iron-depleted rats (Okano et al., 1994) and it was recently demonstrated that vitamin A-fortified salt reduced the prevalence of anaemia in vitamin A- and iron-deficient Moroccan schoolchildren (Zimmermann et al., 2006). Vitamin A supplementation or fortification has been reported to increase Hb level (Muhilal et al., 1988) and to improve iron status indicators (Bloem et al., 1990).

1.3.2 Other micronutrients

Deficiencies of other micronutrients, such as riboflavin, vitamin B12 or folate can also affect iron handling and haematological status (Willoughby and Jewell, 1968; Suharno et al., 1993; Powers, 2003). In the People's Republic of China, a combination of iron, folic acid and riboflavin was more effective than iron plus folic acid alone against anaemia (Ma et al., 2008). However, a recent study conducted among school-aged children in Côte d'Ivoire did not find any relation between riboflavin deficiency and anaemia, although there was a high prevalence of riboflavin deficiency (Rohner et al., 2007). Vitamin B12 has been identified as an important factor of severe anaemia in Africa, whereas the contribution of folate may be less important (van Hensbroek et al., 2011). Furthermore, it is not well established whether these micronutrients play an important role in the aetiology of anaemia in countries highly endemic for multiple species parasitic infections.

1.3.3 Infectious diseases

There is mounting evidence that a substantial part of anaemia, particularly in the tropics, is due to infectious diseases such as malaria and helminthiasis (Crawley, 2004; Brooker et al., 2007; van Hensbroek et al., 2011). Countries in sub-Saharan Africa are endemic for malaria and helminths and cross-sectional surveys carried out in Côte d'Ivoire and elsewhere confirmed that polyparasitism is the norm rather than the exception (Utzinger et al., 1999; Keiser et al., 2002; Raso et al., 2004; Tolentino and Friedman, 2007; van Hensbroek et al., 2011). In Africa, school-aged children are at highest risk of co-infection with *Plasmodium falciparum* and hookworm and this often has an additive negative impact on Hb level (Brooker et al., 2007). Since the contribution of the different parasites to anaemia have been characterised or modelled with data stemming from cross-sectional studies (Carneiro et al., 2006; Ezeamama et al., 2008), there is a serious lack of knowledge concerning the variation of anaemia prevalence in response to seasonal patterns of infectious diseases or to preventive chemotherapy targeting specific parasitic diseases.

1.3.3.1 Malaria

Sub-Saharan Africa accounts for at least 90% of the global burden of malaria with about 1 million dying of malaria every year and millions more suffering clinical consequences of infection (Greenwood and Mutabingwa, 2002; Murray et al., 2012). Children below the age of 5 years represent the most vulnerable population in sub-Saharan Africa (WHO, 2003b). It is estimated that (severe) symptomatic malaria contributes to 18% of childhood deaths in sub-Saharan Africa (Rowe et al., 2006) and causes pregnancy complications (Greenwood, 1997). Evidence suggests that malaria is one of the most important contributing factors to anaemia (WHO, 2002). Both symptomatic and asymptomatic malaria contribute substantially to anaemia in endemic regions (Geerligs et al., 2003; Korenromp et al., 2004). Nevertheless, the estimated contribution of *Plasmodium* to anaemia varies depending on *Plasmodium* prevalence and the source and the choice of a statistical model (Sharp and Harvey, 1980; Menendez et al., 1997; Reyburn et al., 2005; Carneiro et al., 2006). Due to the large number of asymptomatic carriers in children, its relative contribution might be of importance, and hence warrants in-depth studies. Côte d'Ivoire is a highly endemic country where long-lasting insecticidal net (LLIN) coverage rate was among the lowest in a recent survey (Noor et al., 2009). A national campaign, carried out between late 2010 and mid-2011 certainly increased coverage rates.

Anaemia can develop in individuals with chronic infection and low parasitaemia as well as in individuals with acute *P. falciparum* malaria with high parasitaemia. The pathogenic factors involved in malarial anaemia are incompletely understood. However, many mechanisms have been described in *Plasmodium*-infected patients, namely the rupture of parasitized RBC, haemolysis, increased splenic clearance of infected and uninfected RBC, insufficient erythropoiesis, dyserythropoiesis and decreased iron absorption in duodenum (Nagel, 2002; Ekvall, 2003; Chang and Stevenson, 2004; Cercamondi et al., 2010).

1.3.3.2 Parasitic worms

An estimated 2 billion people are infected with soil-transmitted helminths (*Ascaris lumbricoides*, hookworm and *Trichuris trichiura*) and schistosomes (in Africa: *Schistosoma haematobium* and *S. mansoni*) and at least 50% of the 300 million individuals exhibiting signs of disease-related morbidity are school-aged children (WHO 2003a). Soil-transmitted helminths and schistosome infections are highly endemic in sub-Saharan Africa. Data from Côte d'Ivoire suggest that between a third and up to half of the school-aged population is affected by hookworm (Raso et al., 2004; Matthys et al., 2007) and that approximately 40% of the population is infected with either *S. haematobium* or *S. mansoni* or both species concurrently (Steinmann et al., 2006; Schur et al., 2011). Parasitological data from Côte d'Ivoire indicate that polyparasitism is very common (Utzinger et al., 1999; Keiser et al., 2002; Raso et al., 2004). A recent investigation conducted in the Philippines showed that the effect of concomitant high infection by hookworm and *S. japonicum* or *T. trichiura* had a synergistic negative impact on Hb level (Ezeamama et al., 2008). Inversely, the administration of combined albendazole and praziquantel to Tanzanian children living in an area of high endemicity for helminth and schistosome infections significantly improved Hb concentration (Bhargava et al., 2003). In Uganda, the same drug combination produced a 52% reduction in the proportion of cases of anaemia; the most marked improvements occurred among the most heavily infected children (Kabatereine et al., 2007). Although these results stem from different areas, they emphasise that helminths represent an important factor in the course of anaemia.

Moderate-to-heavy infections with hookworm (≥ 2000 eggs/g of stool, EPG) are strongly associated with insufficient Hb level and the risk of anaemia is correlated with infection intensity (Stephenson et al., 1985, Brooker et al., 1999, Friedman et al., 2005). The amount of blood loss in hookworm infections (*Ancylostoma duodenale* and *Necator americanus*) is strongly and linearly correlated with worm load and faecal egg count, and even light infections contribute significantly to anaemia (Stoltzfus et al., 1996, Hotez et al., 2004). *T. trichiura* also causes

intestinal blood loss, and school-aged children with heavy *T. trichiura* infections have a higher prevalence of anaemia than their non-infected counterparts (Ramdath et al., 1995). However, a recent Cochrane review indicated that there is still small evidence about the beneficial effect of community soil-transmitted helminth deworming on Hb concentrations (Taylor-Robinson et al., 2012). The potential mechanisms linking intestinal worms and anaemia include feeding by the worms on host tissues and blood, maldigestion or malabsorption of (micro)nutrients and inflammatory response leading to the production of cytokines, modifying the metabolism and storage of micronutrients (Hall et al., 2008).

Schistosome infections are associated with anaemia (Traore et al., 1998; King et al., 2005a) but the causal relationship is unclear. Potential mechanisms include extra-corporal loss of iron, splenomegaly leading to RBC sequestration, autoimmune haemolysis and anaemia of inflammation, also called anaemia of chronic disease (ACD) (Friedman et al., 2005). The direct causal relation between schistosome infection and anaemia is often difficult to assess, since multiple confounding factors coexist (Koukounari et al., 2006). A meta-analysis with previously published studies indicates that individuals infected with *S. mansoni* manifest significantly greater Hb deficit than non-infected individuals, and specific antischistosomal therapy significantly improved Hb levels (King et al., 2005b). Moreover, a recent randomised placebo-controlled trial showed that anthelmintic treatment (albendazole plus praziquantel) had a modest positive effect on anaemia prevalence in schoolchildren in south-central Côte d'Ivoire (Rohner et al., 2010). Nevertheless, others demonstrated that treatment against schistosomiasis alone failed to improve Hb level (Taylor et al., 2001). Recently, anaemia has been proposed as a subtle-morbidity marker for schistosome infection (Webster et al., 2009). However, the use of Hb concentrations as marker for schistosome infection remains controversial as other factors can affect its concentration.

1.3.3.3 Coinfection

Age-prevalence curves indicate that school-aged children are at the highest risk of malaria and hookworm co-infection, which might exacerbate the anaemia-related malarial disease burden (Brooker et al., 2007). However, the interactions between soil-transmitted helminths and *P. falciparum*, including immunological responses and clinical outcomes of the host, are complex and not yet fully elucidated (Hartgers and Yazdanbakhsh, 2006). While some studies found a negative association between helminth and *Plasmodium* infections (Murray et al., 1978; Brutus et al., 2007; Kung'u et al., 2009; Melo et al., 2010), others suggest that coinfection may exacerbate the morbidity of a single infection (Nacher et al., 2002; Spiegel et al., 2003, Yatich et

al., 2009; Pullan et al., 2011). Different immunological models have been proposed, depending on the stage of infection (acute *versus* chronic) and on *Plasmodium* infection severity (e.g. asymptomatic, clinical, severe or cerebral malaria) (Nacher et al., 2002, Hartgers and Yazdanbakhsh, 2006). Hence, the effect of coinfections on an organism and its immune system and their implications in the development of anaemia remains elusive.

1.3.4 Haemoglobinopathies

Anaemia can also result from inherited disorders of Hb synthesis which prevent efficient binding between globin chains and oxygen. These haemoglobinopathies are the largest group of genetically determined anaemia and occur most frequently in Africa, Asia and the Mediterranean region. These defects in Hb synthesis can result from a single amino-acid substitution in the locus that encode the β -globin peptide (as in sickle cell anaemia) or from a reduced production of α -globin (in α -thalassemia) or β -globin (in β -thalassemia). Although both types of inherited disorders have been associated with protection from malaria, previous studies indicated that major thalasseмии are not prevalent in West Africa, whilst 1% of the population of tropical Africa is affected by sickle cell anaemia (Serjeant, 1992; Taylor et al., 2012).

1.3.5 Other aetiological agents

There are other factors which may have an effect on Hb levels. Several studies investigated the relationship between HIV/AIDS and anaemia (Calis et al., 2008). In these studies, however, anaemia was most often investigated as risk factor for disease progression and death rather than an outcome of HIV. Anaemia is most frequent in patients with AIDS and potential mechanisms include failure of erythropoiesis and chronic inflammation due to frequent bacterial, viral and fungal infections (Bain, 1999). Furthermore, severe anaemia is common in HIV-infected individuals, particularly if they are co-infected with tuberculosis (McKew and Bates, 2011).

Glucose-6-phosphate dehydrogenase (G6PDH) deficiency is another important cause of anaemia, and the only study which investigated this inherited RBC disorder in Côte d'Ivoire found a prevalence of 30.7% among the adult population (Coulibaly et al., 2000). For an accurate diagnosis of G6PDH, genotyping G6PDH alleles is required. HIV diagnosis raises ethical questions since a positive diagnosis might be associated with stigma. Furthermore, HIV-infected individuals require appropriate care and treatment, which might not be easily accessible. Hence, these parameters were not investigated in the present thesis.

1.4 Consequences of anaemia

The consequences of anaemia are manifold and depend on individual factors and on the severity of anaemia. Subtle consequences include reduced oxygen transport and energy metabolism which often cause tiredness, weakness and may decrease working capacity (Haas and Brownlie, 2001). Furthermore, anaemia in infancy might lead to poorer cognition, school achievement, and behavioural problems during childhood, although the confounding effect of poor socioeconomic status or other micronutrient deficiencies cannot systematically be excluded (Grantham-McGregor and Ani, 1999; 2001). In elderly, anaemia has been associated with an increased risk of incident dementia (Peters et al., 2008). The consequences of anaemia also include poor pregnancy outcomes such as premature births and increased peri-natal, infant and maternal mortality (WHO/UNICEF/UNU, 2001).

1.5 Assessment of anaemia and its potential aetiological agents

Anaemia is most often defined according to WHO guidelines although discussions are ongoing whether cut-offs should be adjusted for the African population (Jackson et al., 1983; Beutler and West, 2005). A critical step in the identification and the quantification of the aetiological agents of anaemia is the choice of the diagnostic approach. Indeed, methods and biomarkers available for the diagnosis of parasitological agents and micronutrient status, respectively, have an important leverage on the results. Several methods are available for each parameter, yet the affordability and feasibility in resource-poor settings remain formidable challenges.

1.5.1 Parasitological agents

For *Plasmodium* infection, results from Giemsa-stained blood film examinations, rapid diagnostic tests (RDTs) or polymerase chain reaction (PCR) can give considerably different results (Ndao et al., 2004; Wongsrichanalai et al., 2007). Various methods are also available for the diagnosis of helminth infection and the current 'gold' standard method (e.g. Kato-Katz thick smear examination for *S. mansoni* and soil-transmitted helminths) is not the most sensitive to detect the presence of parasitic worms (Knopp et al., 2009; Coulibaly et al., 2011; Utzinger et al., 2011).

1.5.2 Micronutrient status

Several combinations of the variables age, height and weight can be used to assess the rough prevalence of micronutrients deficiency in infants and children, through the calculation of weight-for-height, height-for-age and weight-for-age Z-scores and their comparison with a reference

population (WHO, 1995). However, these indicators do not provide specific information about the source of malnutrition. Body reserve in minerals and vitamins is generally assessed by biochemical laboratory analyses which directly or indirectly measure the quantity of a given nutrient. Assessing micronutrients status can turn out challenging in population where chronic diseases are endemic as inflammation may bias the measure of micronutrients.

The examination of bone marrow (BM) aspirate, stained with Prussian blue for iron particles, is still considered as the most specific and sensitive method for evaluating iron status although the numerous disadvantages as its high cost, invasiveness and operator-dependant results prevent its frequent use in epidemiological studies. Moreover, recent studies indicate that this method also has its limitations for the diagnosis of ID (Barron et al., 2001; Ganti et al., 2003; Ervasti et al., 2004). The red cell indices mean corpuscular volume (MCV) and mean corpuscular Hb (MCH) are commonly used in the classification of anaemia (Hillman, 1998), although these values are influenced by specific conditions such as haemoglobinopathies and anaemia of inflammation.

The most widely used marker to diagnose ID is plasma ferritin (PF). PF reflects total body iron stores and a concentration below 12 µg/l is usually considered as ID in individuals without inflammation (WHO/UNICEF/UNU, 2001). PF transcription is regulated by cellular iron content through the iron-responsive elements (Torti and Torti, 2002). Furthermore, PF is an acute phase protein whose concentration can be higher than 100 µg/l when inflammation and empty iron stores coexist in the organism (Witte, 1991). High PF concentration has also been associated with *Plasmodium* infection (Stoltzfus et al., 1997), most likely explained by the regulation of ferritin and PF secretion by inflammatory cytokines (Torti and Torti, 2002). Hence, PF is not a sensitive marker of ID in regions with high prevalence of chronic infections or inflammatory diseases (Baynes and Bothwell, 1990). Several correction factors have been proposed to adjust the values of PF for inflammation but there is no consensus yet on their use (Witte, 1991; Thurnham et al., 2010). In parallel to increased ferritin concentrations, inflammation also induces the expression of hepcidin. Hepcidin is a systemic iron-regulatory hormone whose transcription and synthesis are regulated, among others, by iron and inflammatory cytokines (Ganz and Nemeth, 2012). Hepcidin regulates dietary absorption in the duodenum, recycling of iron from macrophages and release of stored iron from hepatocytes by inducing degradation of its receptor, the cellular iron exporter ferroportin (**Figure 1.3**). Although both ferritin and hepcidin are increased in the anaemia of inflammation, the interaction and inter-regulation of both proteins requires further investigation.

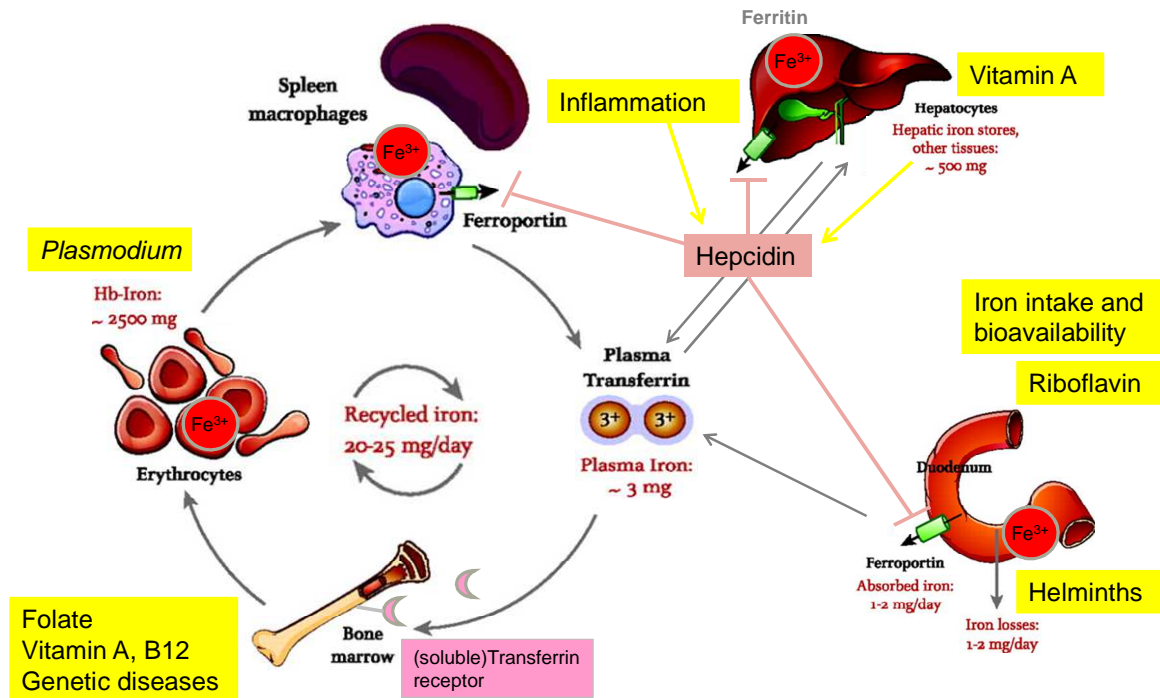


Figure 1.3: Iron homeostasis and regulation by hepcidin.

Hepcidin is a protein secreted by hepatocytes in response to sufficient iron stores or inflammation. Hepcidin inhibits intestinal iron absorption, iron mobilization from hepatocytes and iron recycling from macrophages through its binding to ferroportin and subsequent degradation. This process might cause functional iron deficiency, a state defined as sufficient iron stores which cannot be mobilized for iron-dependent processes (e.g. erythropoiesis). Several factors which can potentially affect iron homeostasis or erythropoiesis are represented by yellow boxes. Modified from Evstatiev and Gasche (2010).

Soluble transferrin receptor (sTfR) is a marker of cellular iron demand more recently discovered and increasingly used in epidemiological studies as marker of ID, particularly in regions where inflammatory diseases are prevalent and in populations affected by chronic diseases (Staubli-Asobayire et al., 2001; Labbe and Dewanji, 2004; Koulaouzidis et al., 2009). Indeed, previous studies suggest that inflammation does not affect sTfR concentration (Ayoya et al., 2010). However, a recent study conducted in Kenyan preschool-aged children showed that sTfR concentrations were significantly higher in infants with inflammation and adjusting sTfR concentrations for inflammation significantly modified the estimated prevalence of ID (Grant et al., 2012). In addition, expanded erythropoiesis, including haemolytic and malarial anaemia, may increase sTfR concentrations (Verhoef et al., 2001). However, a study conducted in Beninese women with asymptomatic malaria found higher concentrations of sTfR after the administration of Malarone (250 mg atovaquone + 100 mg proguanil hydrochloride), suggesting a suppression of erythropoiesis elicited by chronic *Plasmodium* infection (Cercamondi et al., 2010). The transcription of sTfR is regulated by iron-responsive elements, as well. However, in contrary to

the well-known and well-described effects of inflammation on hepcidin and ferritin (Torti and Torti, 2002; Ganz and Nemeth, 2012), the interaction between inflammatory cytokines and sTfR expression remains to be fully elucidated (Figure 1.3).

Another biomarker of ID frequently used in epidemiological studies is zinc protoporphyrin (ZPP). When iron supply does not reach erythropoiesis iron demand, zinc is incorporated into protoporphyrin and ZPP is produced instead of haeme. The ZPP/haeme ratio can easily be measured by haematofluorometry and has potential as a screening test because it can detect ID at stages preceding anaemia (Labbe et al., 1999). In unfrozen washed erythrocytes samples from healthy people without ID, ZPP is ≤ 40 $\mu\text{mol/haeme}$ (Hastka et al., 1992). However, it is still unclear to what extent ZPP is influenced by inflammation, infection and thawing and hence there is still no consensus on the exact cut-off defining ID in settings where inflammatory diseases are widespread or in samples which have been thawed once (Zimmermann et al., 2005). Hence quantifying and identifying the main causes of anaemia in a setting with high prevalence of infectious or inflammatory diseases remains challenging, and caution is required when interpreting the results.

1.6 Prevention and control of anaemia in the humid tropics

As anaemia is multifactorial in sub-Saharan Africa and elsewhere, an integrated approach targeting the main aetiological agents is mandatory to better understand the main drivers of anaemia and to ultimately decrease the burden related to anaemia in the humid tropics (WHO/UNICEF, 2004). Preventive and control measures against malaria consist mainly of indoor residual spraying (IRS), LLINs, intermittent-preventive treatment (IPT) of malaria and prompt diagnosis and treatment of malaria with artemisinin-based combination therapies (ACTs) (WHO, 2011). The potential development and spread of resistances at the vector and parasite levels are serious challenges which are to be faced and adequately addressed in the years to come (The malERA Consultative Group on Drugs, 2011; The malERA Consultative Group on Vector Control, 2011). WHO guidelines recommend regular administration of anthelmintic drugs to control helminthiasis (WHO, 2004). Furthermore, community-led total sanitation and improved access to clean water are important complementary interventions (Bartram and Cairncross, 2010; Dongre et al., 2011). Non-targeted iron supplementation in malaria-endemic areas is currently not recommended since this intervention turned out harmful in iron-replete children (Sazawal et al., 2006). Iron fortification may be an alternative dietary approach to fight against ID in infection-endemic settings, but the inhibitory effect of *Plasmodium* infection on

intestinal iron absorption makes the development of an efficient iron-fortified food difficult (Cercamondi et al., 2010; Rohner et al., 2010). Moreover, health and nutritional education at different levels should be considered in order to achieve a sustainable impact on the burden of anaemia. Importantly, decentralization and strengthening of health system, with active community involvement, seem to be the way forward in an era where major parasitic infections are being controlled and the next logical step consists of the elimination of the disease (WHO, 2012).

1.6.1 The contribution of the social sciences in the prevention and control of anaemia

Social sciences are broadly integrated in epidemiological studies for their decisive role in situation analysis aiming to develop local and national programme strategies (Brooker et al., 2000; Thompson and Khan, 2003; Banu Rekha et al., 2009). Situation analyses not only entail defining the epidemiology of the disease, but also require to assess health and economic situation of the country, and to investigate people's perceptions and behaviours towards the disease and potential interventions.

Previous socio-anthropological studies done in sub-Saharan Africa about anaemia were limited on knowledge, attitudes, practices and beliefs (KAPB) of one specific population group on a single aetiological agent of anaemia, like malaria (Beiersmann et al., 2007; Esse et al., 2008; Ouattara et al., 2011), soil-transmitted helminths (Acka et al., 2010), ID (Galloway et al., 2002) or sickle-cell trait (Wonkam et al., 2006). Others investigated local concepts and behaviours associated with malaria-related illnesses, ID or tuberculosis (Galloway et al., 2002; Ahorlu et al., 2005; Coulibaly, 2010). However, local concepts about blood and various anaemia-related illnesses and their relation to people's behaviours have never been investigated among different ethnic groups in Côte d'Ivoire although this kind of information is crucial in the development of local intervention strategies aiming to decrease the burden of anaemia.

1.7 Study area

Côte d'Ivoire is a country situated in West Africa whose southern boundary follows the Gulf of Guinea (**Figure 1.4**). About 20 million people live in Côte d'Ivoire with a quarter of the population living below the international poverty line of US\$ 1.25 per day (UNDP, 2008). Large-scale production of coffee and cocoa for the international markets, commencing in the 1960s and

accompanying fast economic development, gave Côte d'Ivoire the nickname “locomotive” of West Africa.

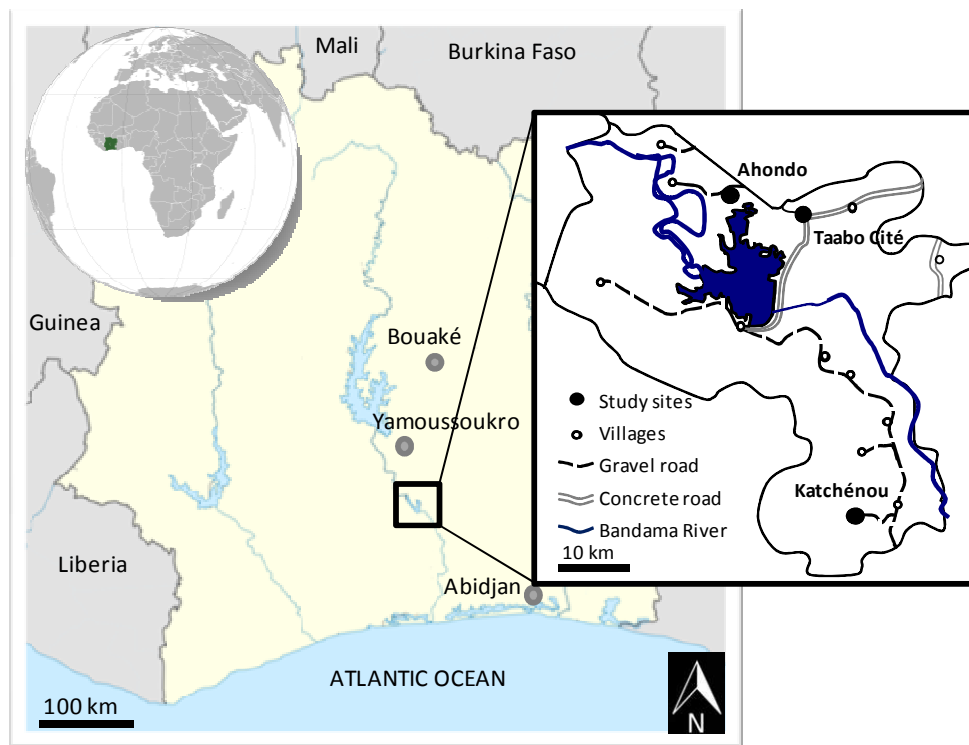


Figure 1.4: Study area.

Côte d'Ivoire is situated in West Africa. The Taabo HDSS is located in south-central part of the country, in close proximity to the Lake Taabo. The longitudinal monitoring took place in three localities of Taabo HDSS: Taabo Cité, the unique small town of the area; Ahondo, a village situated in close proximity to Lake Taabo, and Katchénou, a hamlet later on designated as village. Adapted from <http://www.acor-salesforce.com/> and <http://www.mapanddata.com/carte-de-la-cote-d-ivoire-contours-provinces.html>.

However, the economic crisis Côte d'Ivoire went through in the 1980s and socio-political unrest and armed conflict that the country witnessed since a coup d'État in 1999, prevented the country to keep its good economic developmental prospects. With <1 hospital bed/1,000 people and <1 health centre/10,000 inhabitants, the health system of Côte d'Ivoire is among the weakest health systems throughout the world (World Bank, 1996; Kouassi-Gohou et al., 2009).

The field work of this PhD was conducted in the Taabo health demographic surveillance system (Taabo HDSS), situated in the transition zone from rainforest to Savannah in the V-Baoulé of south-central Côte d'Ivoire (Figure 1.4). The climate is tropical with two rainy seasons, one from April to July and the second from September to October. The Taabo HDSS has been

established in 2008, thanks to generous financial support from FAIRMED¹. Some 40,000 people in over 8,500 households are under continuous health and demographic surveillance. The FAIRMED-supported interventions in the Taabo HDSS aim to improve the health and wellbeing of the population. Specifically, interventions are targeting malaria and neglected tropical diseases, as these have been identified as key public health challenges in the study area by different stakeholders. In March 2012, the Taabo HDSS has been officially recognized and integrated to the International Network for the Demographic Evaluation of Populations and Their Health in Developing Countries (INDEPTH²) as a member site.

Every household is visited once every 3-4 months and in- and out-migrations, births and death, including causes of death according to verbal autopsies, are registered (**Figure 1.5**). The site of the Taabo HDSS covers the area around the man-made lake of Taabo, constructed in the late 1970s (N'Goran et al., 1997). Villagers are mainly engaged in subsistence farming of cassava, plantains and yams. Some people make a living from fishing in Lake Taabo. Coffee and cacao are the predominant cash crops of the study area.

Depending of the region, staple food in Côte d'Ivoire consists notably of cassava, plantain, rice, yams, dried smoked fish, chicken and fruits. Tilapia, catfish and prawns are found in the Lake Taabo and the Bandama River, situated in our study area, and are consumed occasionally (Roche and Tidou, 2009). Local diet does often not cover the estimated average requirement for micronutrients and this may represent an important risk factor for the development of anaemia (Wegmüller et al., 2006).

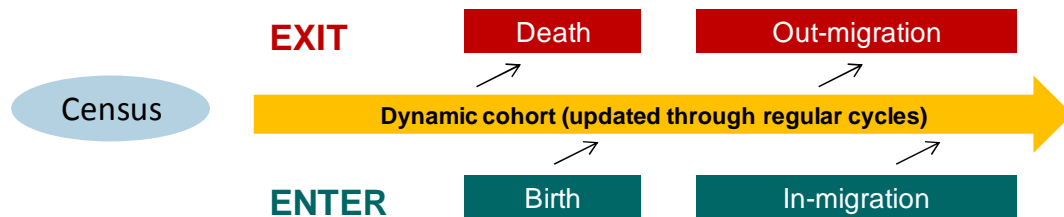


Figure 1.5: Functioning of a health demographic surveillance system (HDSS).

Source: www.indepth.com; accessed 28 June 2012

¹FAIRMED (previously known as Aide aux Lépreux Emmaüs Suisse, ALES in short) is a Swiss non-governmental organization (NGO) which helps in the implementation of projects aiming to procure medical care to the poorest of the poor.

²INDEPTH is a global network of members who conduct longitudinal health and demographic evaluation of populations in low- and middle-income countries. INDEPTH aims to strengthen global capacity for health and demographic surveillance systems (HDSS), and to mount multi-site research to guide health priorities and policies in low- and middle-income countries, based on up-to-date scientific evidence.

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2. Goal and specific objectives

The PhD thesis presented here is part of a 3-year project entitled “Aetiology, prevention and control of anaemia in sub-Saharan Africa”, funded by the Swiss National Science Foundation (Joint Research Projects) and conducted in collaboration with the Institute of Food, Nutrition and Health of the ETH Zurich and the Université de Cocody and the Centre Suisse de Recherches Scientifiques en Côte d’Ivoire (CSRS). This Joint Research Project pursued the following three specific objectives: (i) to monitor longitudinally anaemia in three population cohorts, (ii) to investigate changes in iron metabolism in relation to *Plasmodium* and helminth infections using a stable isotope technique; and (iii) to assess the effect of iron fortification and intermittent preventive treatment (IPT) of malaria on anaemia in infants. My PhD thesis mainly focuses on the monitoring of anaemia in three population cohorts with an overall goal and a set of specific objectives.

The overarching goal of my PhD thesis is to deepen the understanding of the aetiology of anaemia in a primarily rural setting of south-central Côte d’Ivoire, where some control measures against malaria, soil-transmitted helminthiases, schistosomiasis and other neglected tropical diseases are in place, which might lead to suggestions on how to better prevent and control anaemia.

In order to achieve this aim, the following four specific objectives were pursued

- (i) To investigate the local epidemiology of anaemia, micronutrient deficiencies, malaria and helminth infections in three age groups. To determine the prevalence and severity or intensity of anaemia, micronutrients deficiency and parasitic infections among infants (6-23 months), early school-aged children (6-8 years) and young women (15-25 years) in the Taabo HDSS.
- (ii) To elucidate the main causes of anaemia. To determine which socio-demographic, parasitic and nutritional parameters are associated with anaemia in infants, young school-aged children and young women and investigate how anaemia varies over time in relation to the aforementioned parameters in a primarily rural setting of West Africa through a 14-month prospective monitoring with repeated cross-sectional surveys and specific interventions according to participants’ health status (**Figure 2.1**). This objective includes the investigation of potential interactions between multiple parasitic infections (e.g. *Plasmodium* and hookworms).
- (iii) To assess the relation of inflammation and *Plasmodium* infection with iron status

biomarkers. To determine the association between inflammation and *Plasmodium* infection, and sTfR and PF concentrations in different age groups.

(iv) To study local concepts of various anaemia-related illnesses, their relationship with people's behaviours and potential public health implications. To deepen our knowledge of local concept of blood and anaemia in three settings of the Taabo HDSS and to assess their relation with risk-related and help-seeking behaviours.

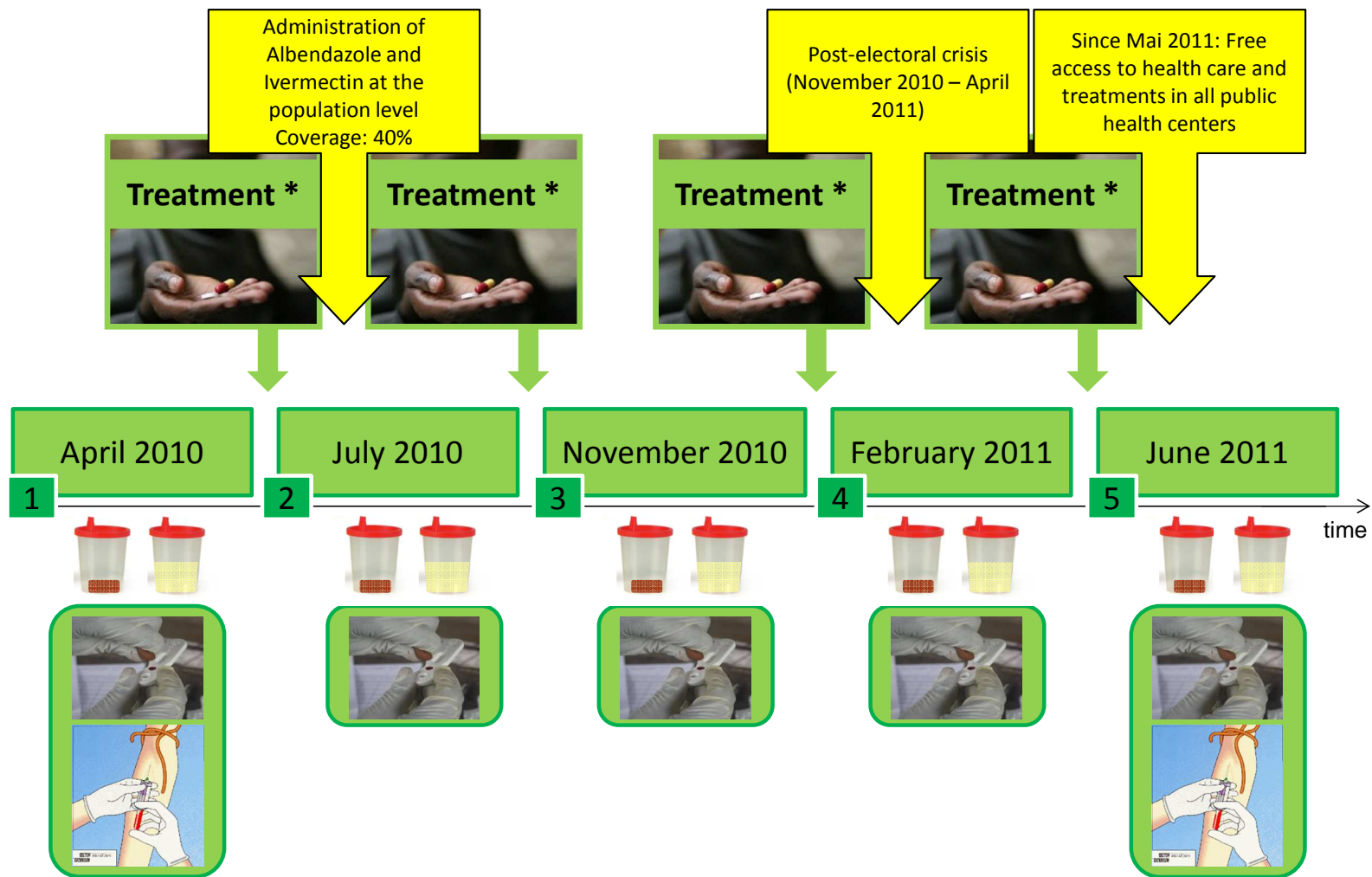


Figure 2.1: Timeline of the 14-month prospective longitudinal monitoring conducted in three cohorts (infants, school-aged children and young women) in Taabo HDSS between April 2010 and June 2011.

* Participants found positive for helminth, schistosome or who presented with clinical malaria (tympenic temperature $>38^{\circ}\text{C}$ and positive RDT) or severe anaemia (Hb $< 8\text{g/dl}$) received adequate treatment and were referred to the health system.

3. Aetiology of anaemia among infants, school-aged children and young non-pregnant women in different settings of south-central Côte d'Ivoire

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3.1 Abstract

Anaemia affects one quarter of the world's population, but its aetiology remains poorly understood. We determined the prevalence of anaemia and studied underlying risk factors in infants (6-23 months), young school-aged children (6-8 years), and young non-pregnant women (15-25 years) in south-central Côte d'Ivoire. Blood, stool and urine samples were subjected to standardized, quality-controlled methods. We found high prevalences of anaemia, malaria, inflammation and deficiencies of iron, riboflavin and vitamin A, but low prevalences and intensities of soil-transmitted helminth and schistosome infections. Multivariate regression analysis revealed significant associations between anaemia and *Plasmodium falciparum* for infants, inflammation for school-aged children and cellular iron deficiency for both school-aged children and non-pregnant women. Women with riboflavin deficiency had significantly lower odds of anaemia. Our findings call for interventions to protect infants from malaria, improved intake of dietary iron, better access to health care and health education.

3.2 Introduction

It is currently estimated that anaemia affects one quarter of the world's population. Most of this burden occurs in developing countries, particularly among preschool-aged children and women of reproductive age (McLean et al., 2009). Iron deficiency (ID) is recognized as the primary cause of anaemia worldwide, yet the aetiology of anaemia is multifactorial, including nutritional habits, bioavailability of micronutrients, parasitic infections (e.g., malaria and helminth infections), inflammation and genetic factors (Tolentino and Friedman, 2007). In addition to tiredness and impaired cognitive performance, the consequences of anaemia include reduced educational achievement and work capacity, increased mortality and morbidity from infectious diseases and poor pregnancy outcomes (WHO/UNICEF/UNU, 2001). In Côte d'Ivoire, previous studies estimated that more than 40% of the population is affected by anaemia (Staubli-Asobayire et al., 2001; Wegmüller et al., 2006; Rohner et al., 2010).

The prevention and control of anaemia is complex and, depending on the setting, might require the implementation of a set of control measures. Iron fortification, long-lasting insecticidal nets (LLINs), intermittent preventive treatment (IPT) of malaria and the regular administration of anthelmintic drugs can be effective strategies to decrease the prevalence of anaemia in developing countries (ter Kuile et al., 2003; Tolentino and Friedman, 2007; Rohner et al., 2010). In Côte d'Ivoire, after one decade of socio-political unrest, armed conflict and war (Bonfoh et al., 2011), new efforts are getting underway to improve people's health and wellbeing. It is important to characterise the baseline situation of anaemia in the most vulnerable population groups to serve as a benchmark for monitoring progress now that new control initiatives are being implemented.

Here, we report the results from a baseline cross-sectional survey carried out in the recently established Taabo health demographic surveillance system (Taabo HDSS) in south-central Côte d'Ivoire (Becker et al., 2011). The goal was to determine the prevalence of anaemia and to study the main risk factors for three population groups (i.e., infants, children at early school age and young non-pregnant women). These findings have been instrumental for designing control interventions (e.g., community-based anthelmintic treatment, distribution of LLINs) and study participants will be followed longitudinally.

3.3 Materials and Methods

3.3.1 Ethical considerations

The study protocol was approved by the institutional research commissions of the Swiss Tropical and Public Health Institute (Swiss TPH; Basel, Switzerland, reference no. FK 96) and ETH Zurich (reference no. EK 2009-N-19). Ethical approval was granted by the ethics committee of Basel (EKBB, reference no. 252/09) and Côte d'Ivoire (reference no. 1086 MSHP/CNER). Study investigators were covered by liability insurance (GNA Assurance; Abidjan, Côte d'Ivoire, policy no. 30105811010001). Village chiefs, participants and parents/guardians of children were informed about the purpose and procedures of the study. Written informed consent (or fingerprints of illiterate people) was obtained from study participants and the parents/guardians of children. Participants accepted to take part in a longitudinal monitoring (5 sampling time points, once every 3-4 months), but could withdraw from the study at any time without further obligations. Suspected clinical malaria (i.e., positive rapid diagnostic test (RDT) and tympanic temperature $>38^{\circ}\text{C}$), severe anaemia (i.e., haemoglobin (Hb) <8 g/dl according to the national cut-off defining severe anaemia) and helminth infections were treated according to national guidelines.

3.3.2 Study setting

The study area lies in the transition zone from rainforest to Savannah in the V-Baoulé of south-central Côte d'Ivoire (**Figure 3.1**). There are two rainy seasons; a long one lasting from April to July and a shorter one in September and October. Villagers are mainly engaged in subsistence farming of cassava, plantains and yams. Coffee and cacao are the predominant cash crops. Some men make a living from fishing in Lake Taabo.

This study was carried out in the Taabo HDSS. The site covers the area around the man-made lake of Taabo impounded in the late 1970s (N'Goran et al., 1997). We purposely selected individuals from three settings that are representative of the main social-ecological contexts in the Taabo HDSS: Taabo Cité (the only small town), Ahondo (one of the 13 main villages, located in close proximity to Lake Taabo) and Katchénou (initially considered one of over 100 small hamlets, later on designated as a village). According to the April 2010 census, there were 6,813 people living in Taabo Cité.

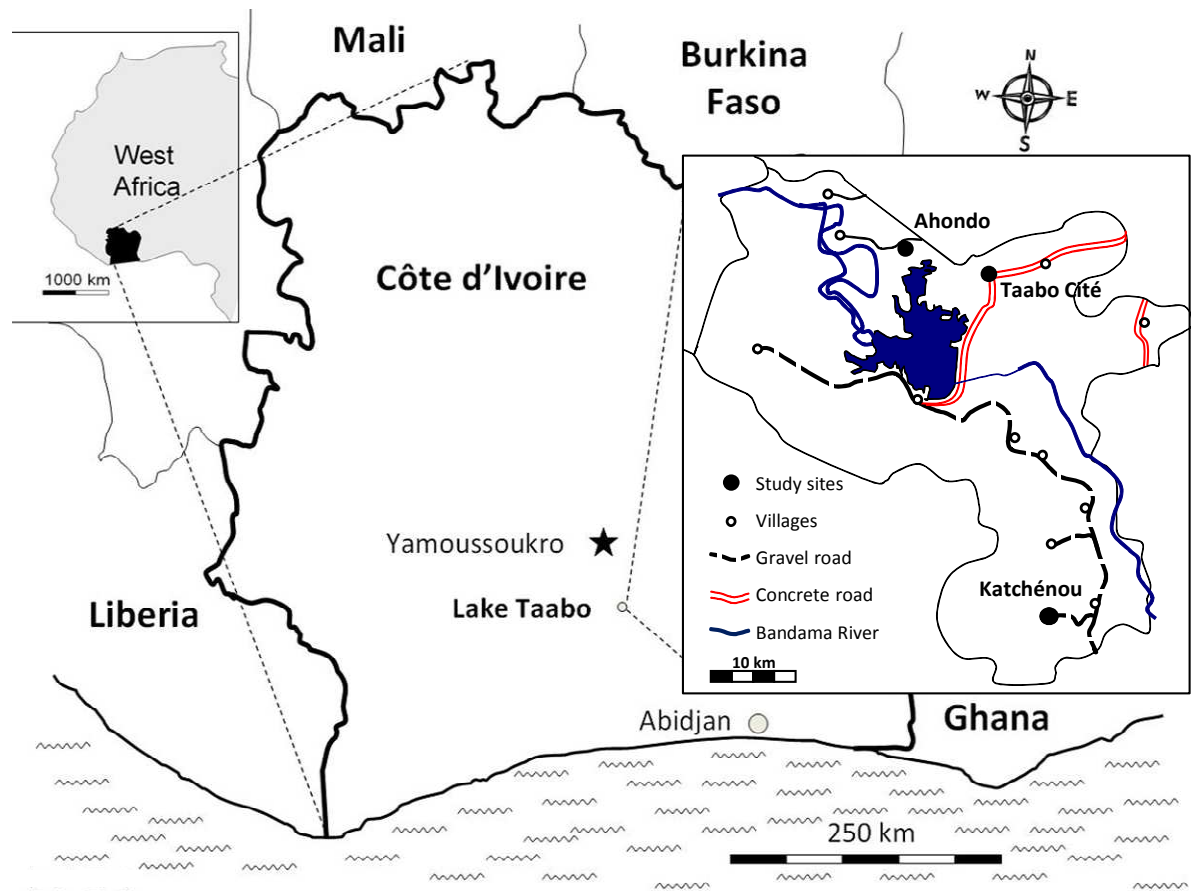


Figure 3.1: Study sites.

The study was embedded in the recently established Taabo health and demographic surveillance site (Taabo HDSS), located in south-central Côte d'Ivoire. Taabo HDSS covers the area around Lake Taabo. This survey was conducted in three settings: Taabo Cité, the only small town; Ahondo, one of 13 main villages in close proximity to Lake Taabo; and Katchénou, at the time of the survey considered as one of over 100 small hamlets, situated 55 km south of Taabo Cité. Modified from Glinz and colleagues (Glinz et al., 2010).

This small town is located approximately 170 km north-west of Abidjan. There is one small hospital with 12 beds. The population in Ahondo and Katchénou are 2,230 and 693, respectively. Ahondo has a health dispensary and is situated 14 km west of Taabo Cité. Katchénou is located 55 km south of Taabo Cité, with no health facility at the time of the current study.

3.3.3 Sample size calculation

The intended sample size at enrolment was 137 individuals in each of the three age-groups, which would allow an accurate estimation of the prevalence of anaemia in the selected groups, with a 9% error margin (Lemeshow et al., 1990). Confidence level was set to 95% and the estimated values for the proportion of anaemia in our population were 60% for infants and school-aged children and 40% for non-pregnant women (Staubli-Asobayire et al., 2001; Wegmüller et al., 2006; Rohner et al., 2010). The sample size was calculated considering a drop-out rate of 20%.

3.3.4 Study design and participants

We report the results from the baseline cross-sectional survey of a 14-month monitoring activity, carried out in April 2010 in the three study settings. The three age-groups included were (i) infants aged 6-23 months; (ii) children at early school age (6-8 years); and (iii) young non-pregnant women aged 15-25 years. In Ahondo and Katchénou, all people within these age ranges were invited to participate. In Taabo Cité, in order to get a similar sample size, 120 infants, 90 school-aged children and 90 young women were randomly selected from the existing database available at the Taabo HDSS.

3.3.5 Field and laboratory procedures

One week prior to our cross-sectional survey, field enumerators went to all households selected for the study and explained the purpose, procedures and potential risks and benefits of the planned work. On the evening before the first sampling day, each participant was given two plastic containers and invited to return filled containers with a fresh morning stool and a urine sample. On sampling day, the purpose of the study and the longer term monitoring were again explained and arising questions by the people were answered. Those individuals who were interested to participate were asked to sign a written informed consent or to give a fingerprint (illiterates), followed by stool and urine collection. Unique identification numbers were assigned to participating individuals and these numbers were used in subsequent surveys.

Participants' height (to the nearest cm) and weight (to the nearest 0.5 kg) were recorded. Temperature was measured by a digital, battery-powered ear thermometer (to the nearest 0.1°C). Subsequently, finger-prick blood was collected from each participant. The presence of *Plasmodium falciparum* was determined using a rapid diagnostic test (ICT ML01 malaria Pf kit; ICT Diagnostics, Cape Town, South Africa). The determination of *Plasmodium* species was done with a thin blood film and parasitaemia assessed with a thick blood film. Hb quantification was done with a portable HemoCue Hb 301 device (HemoCue AB; Ängelholm, Sweden). Finally, a minimum of 5 ml venous blood was collected in heparin-coated tubes that were immediately put in a cool box containing ice.

Blood, stool and urine samples were transferred to the Taabo hospital laboratory. Blood samples were centrifuged, aliquoted and kept at -20°C before transfer to Abidjan and, finally, to Switzerland. Duplicate Kato-Katz thick smears were prepared from each stool sample (Katz et al., 1972). Slides were allowed to clear for 30-45 min before examination under a microscope for the presence of *Schistosoma mansoni* and soil-transmitted helminth (*Ascaris lumbricoides*, hookworm and *Trichuris trichiura*) eggs by laboratory technicians. The

number of eggs was recorded for each helminth species separately. Urine samples were subjected to a filtration method (WHO, 1991). In brief, urine specimens were shaken and 10 ml were gently pressed through a small-meshed filter. One drop of Lugol solution was added on the filter before the slides were quantitatively examined for *S. haematobium* eggs under a microscope by laboratory technicians. For quality control, 10% of the slides were re-examined by a senior technician and, in case of discrepancies, the results were discussed with the technicians and the corresponding slides re-examined until agreement was found.

3.3.6 Venous blood examination

Whole blood was screened for haemoglobinopathies within 1 week of blood sampling by using electrophoresis on cellulose acetate membranes (Schneider, 1974). Riboflavin was measured by the erythrocyte glutathione reductase activity coefficient (EGRAC) assay, using a modification of the method of Dror, Stern and Komarnitsky (Dror et al., 1994). Previous validation studies done in our laboratories revealed inter- and intraassay coefficient of variation (CV) of the EGRAC method of 3% and 4%, respectively. Although EGRAC cut-off values for defining riboflavin deficiency are still being debated, we used cut-off values >1.4 indicating clear deficiency (Jackson et al., 1983). Ferritin, soluble transferrin receptor (sTfR), retinol binding protein (RBP), α 1-acid glycoprotein (AGP) and C-reactive protein (CRP) were measured with a sandwich enzyme-linked immunosorbant assay (ELISA) (Erhardt et al., 2004). Serum retinol (SR) was measured by high pressure liquid chromatography (HPLC; Merck-Hitachi, Tokyo, Japan) according to Tanumihardjo, Permaesih and Muhilal (Tanumihardjo et al., 2004) with reference material from the National Institute of Standards and Technology (Gaithersburg, MD, USA).

3.3.7 Statistical analysis

Parasitologic data were entered twice in Microsoft Access version 10.0 (2007 Microsoft Corporation). Serological data were entered in Microsoft Excel version 10.0 (2007 Microsoft Corporation). Double-entered datasets were compared using EpiInfo version 3.4.1 (Centers for Disease Control and Prevention; Atlanta, GA, USA) and discrepancies removed according to original records. Data were analysed using STATA version 10 (StataCorp.; College Station, TX, USA). For multivariate logistic regression, only those individuals with complete data records were considered.

For each individual, the arithmetic mean of the helminth species-specific egg counts from the Kato-Katz thick smears was calculated and multiplied by a factor 24 to obtain a standardized measure of infection intensity (i.e., eggs per gram of stool, EPG). Hb thresholds used to define anaemia were 11.0 g/dl for infants aged 6-23 months, 11.5 g/dl for children aged 6-8

years and 12.0 g/dl for non-pregnant women aged ≥ 15 years, according to WHO guidelines (WHO/UNICEF/UNU, 2001). Anaemia was classified into moderate and severe using Hb cut-offs of < 9.0 g/dl and < 7.0 g/dl, respectively. Storage iron depletion was defined as ferritin < 12 $\mu\text{g/l}$ for infants without inflammation and < 15 $\mu\text{g/l}$ for children and women without inflammation. For participants with AGP > 1 g/l or CRP > 10 mg/l, storage iron depletion was defined as ferritin < 30 $\mu\text{g/l}$ (WHO/UNICEF/UNU, 2001). Cellular ID was defined as sTfR > 8.5 mg/l (Cook et al., 1993). Acute and chronic inflammation were respectively defined as CRP > 10 mg/l and AGP > 1 g/l. SR < 0.7 $\mu\text{mol/l}$ indicated vitamin A deficiency (VAD) (WHO, 2011). Since only 62 infants provided enough venous blood to quantify retinol through HPLC, RBP < 0.825 $\mu\text{mol/l}$ was used as surrogate for VAD in infants (Gorstein et al., 2008). Since inflammation influences the concentration of many nutritional biomarkers (Thurnham et al., 2003, Wang et al., 2010), the estimated prevalence of micronutrient deficiencies was based exclusively on values from participants with CRP values ≤ 10 mg/l, excluding the 55 participants with acute inflammation.

Multivariate logistic regression was used for determining variables significantly associated with anaemia, using available WHO criteria. Odds ratios (ORs) were calculated, including 95% confidence intervals (CIs) and Wald-test p-value. Candidate explanatory variables for the multivariable logistic regression model were age, sex (for infants and school-aged children), school attendance (for children), type of activity (for women), soil-transmitted helminth, *Schistosoma* and *Plasmodium* infection, storage iron depletion, cellular ID, riboflavin deficiency, VAD (based on retinol values for women and school-aged children and on RBP values for infants), acute inflammation and chronic inflammation. A backward stepwise multivariate logistic regression with locality as random effect was computed for each age group, removing non-predicting covariates up to a significance level of 0.2. The remaining covariates were included into the final models and the variables with a significant p-value are reported.

3.4 Results

3.4.1 Study cohort and compliance

Overall, 732 individuals in Taabo Cité, Ahondo and Katchénou were eligible and hence invited to participate in the baseline cross-sectional survey (**Figure 3.2**). Written informed consent and stool and urine samples were provided by 407 individuals who were included in subsequent analyses. Complete parasitologic data were obtained for 375 individuals.

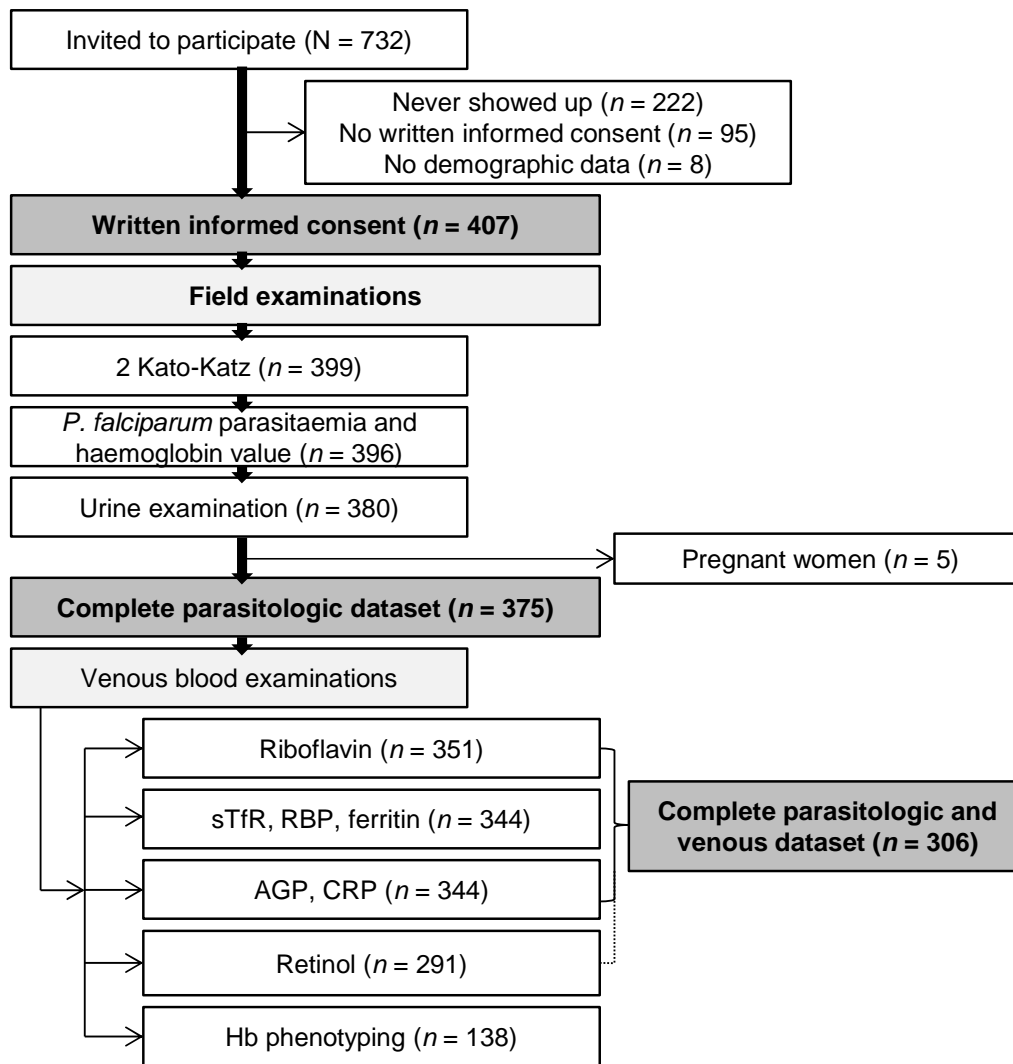


Figure 3.2: Study participation and compliance at baseline.

Diagram detailing the study participation of infants (6-23 months), school-aged children (6-8 years) and non-pregnant women (15-25 years) from Taabo Cité, Ahondo and Katchénou, in April 2010. Individuals who provided at least one urine and/or one stool sample were considered for further analyses. Blood samples from individuals with a complete parasitologic dataset were analysed for nutrition parameters and inflammatory markers. Pregnant women were not considered for the final analyses. AGP, alpha-glycoprotein; CRP, C-reactive protein; Hb, haemoglobin; RBP: retinol binding protein; sTfR, soluble transferrin receptor.

Venous blood samples obtained from individuals with complete parasitologic data were subjected to detailed laboratory work-up. However, due to insufficient quantities of blood, EGRAC could only be determined in 351 blood samples. ELISA and HPLC analyses were carried out using plasma samples from 344 and 291 individuals, respectively.

A total of five women gave birth to a child within the following 9 months and hence were excluded in further analyses. Finally, 306 participants had complete data pertaining to parasitic infection, nutrition and inflammation status. We intended to take one third of the blood samples collected in each locality to assess the prevalence of haemoglobinopathies in our population. Hence, a sub-sample of 138 blood samples was used for Hb phenotyping.

3.4.2 Attrition analysis

Given the low number of people with complete datasets ($n = 306$, 41.8%) an attrition analysis was performed for each age group to compare the characteristics of people who dropped out of the study with those who had complete parasitologic and venous blood data. No significant difference was found for sex (infants and school-aged children) and median age (for school-aged children and women). Infants with complete data records were significantly older than those who dropped out (Wilcoxon rank sum test $P = 0.008$).

3.4.3 Population characteristics

Population characteristics of participants with complete parasitologic data ($n = 375$), stratified by study setting, are presented in **Table 3.1**. More than 70% of the 6- to 8-year-old children were attending school in Taabo Cité, whereas one out of two children did not attend school in Ahondo and Katchénou. Women in the three localities showed marked differences regarding educational attainment, occupation, number of children and matrimonial status. Women were predominantly working outside the home and less than 10% had more than three children, which was to be expected as our inclusion criteria was age between 15 and 25 years.

Table 3.1: Population characteristics according to Taabo HDSS data, stratified by study setting and age group (n = 375).

	Taabo Cité		Ahondo		Katchénou		Difference	
	n	%	n	%	n	%	χ^2	P-value
Age-group								
Infants (6-23 months)	56	37.1	39	34.2	33	30.0		
Children (6-8 years)	61	40.4	46	40.4	51	46.4		
Women (15-25 years)	34	22.5	29	25.4	26	23.6	1.83	0.767
Sex (female)¹	60	51.3	38	44.7	40	47.6	0.87	0.647
Occupation								
Attending school ²	44	72.1	26	56.5	28	54.9	4.33	0.114
Never attended school ³	8	22.9	13	41.9	18	71.4		
Preschool ³	0	0.0	0	0	0	0.0		
Primary school ³	12	37.1	12	41.9	7	25.0		
Secondary school ³	14	40.0	1	6.5	1	3.6		
High school ³	0	0.0	0	0	0	0		
Muslim school ³	0	0.0	3	9.7	0	0	30.54	<0.001
Working ³	14	42.9	19	61.3	24	92.9		
Student ³	13	37.1	6	22.6	1	3.6		
Housekeeper ³	7	20.0	4	16.1	1	3.6	16.91	0.002
Residence in the village								
< 6 months ⁴	3	3.2	2	2.7	0	0		
6 months-1 year ⁴	7	7.4	11	14.7	2	2.6		
> 1 year ⁴	85	89.5	62	82.7	75	97.4	10.17	0.038
Family								
No children ³	24	70.6	12	41.4	3	11.5		
1-3 children ³	8	25.5	17	58.6	22	84.6		
>3 children ³	2	5.9	0	0	1	2.9	24.1	<0.001
Single ³	27	79.4	13	44.8	3	11.5		
With partner ³	3	8.8	8	27.6	14	53.9		
Married ³	3	8.8	8	27.6	9	34.6		
Other ³	1	2.9	0	0	0	0	31.27	<0.001

¹ considered for infants and children (n = 286).² considered for children only (n = 158).³ considered for women only (n = 89).⁴ considered for children and women (n = 247).

3.4.4 Prevalence of anaemia, stratified by age and setting

The overall prevalence of anaemia among participants with complete parasitologic data was 58.4%, with no difference in the prevalence and severity of anaemia according to study setting. As shown in **Table 3.2**, infants had the highest prevalence of anaemia (78.1%). Among them, 61%, 32% and 7% had mild, moderate and severe anaemia. In school-aged children and women, slightly less than 50% were anaemic. There was no case of severe anaemia in children and only one woman suffered from severe anaemia.

Table 3.2: Prevalence and severity of anaemia (n = 375).

	Infants (n = 128)		Children (n = 158)		Women (n = 89)	
	cases	%	cases	%	cases	%
Anaemia ¹	100	78.1	74	46.8	45	47.9
Mild	61	61.0	70	94.6	42	93.3
Moderate ²	32	32.0	4	5.4	2	4.4
Severe ³	7	7.0	0	0	1	2.2

	Taabo Cité (n = 151)		Ahondo (n = 114)		Katchénou (n = 110)	
	cases	%	cases	%	cases	%
Anaemia ¹	76	50.3	72	63.2	71	64.6
Mild	58	76.3	60	83.3	55	77.6
Moderate ²	17	22.4	9	12.5	12	16.9
Severe ³	1	1.3	3	4.2	4	5.6

¹ Anaemia is defined as Hb < 12 g/dl for women; Hb < 11.5 g/dl for children; Hb < 11 g/dl for infants.

² Moderate anaemia is defined as Hb < 9 g/dl.

³ Severe anaemia is defined as Hb < 7g/dl.

3.4.5 Prevalence of parasitic infections, micronutrient deficiencies and haemoglobinopathies

The prevalence of parasitic infection, inflammation and micronutrient deficiency in each setting, stratified by age, is presented in **Figure 3.3**. *P. falciparum* was highly prevalent throughout. However, RDT results revealed significantly higher prevalence in Katchénou (75%) compared to Ahondo (54%) and Taabo Cité (33%) ($\chi^2 = 45.90$; $P < 0.001$). The highest prevalence of *Plasmodium* infection was found in school-aged children (74%), whereas the respective prevalence in infants (39%) and non-pregnant women (30%) were considerably lower ($\chi^2 = 56.06$; $P < 0.001$). Among participants found positive for *P. falciparum*, 27% of the infants, 9% of the school-aged children and 7% of the non-pregnant women harboured > 5,000 parasites/ μ l of blood.

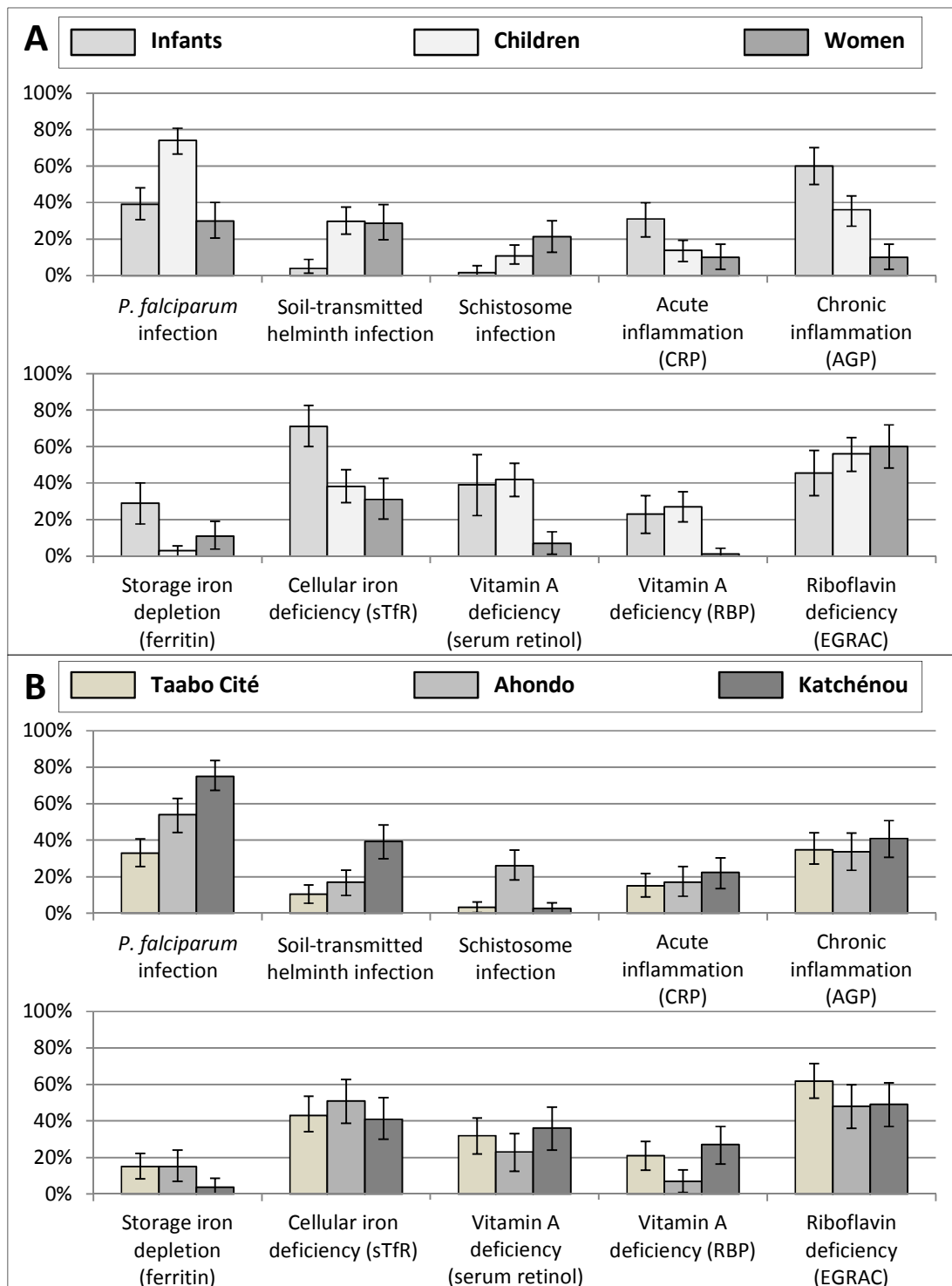


Figure 3.3: Prevalence of parasitic infections, micronutrient deficiency and inflammation stratified by age groups and settings.

A. Prevalence of *P. falciparum*, soil-transmitted helminth and schistosome infection (n = 375), acute inflammation (n = 251), chronic inflammation (n = 251), storage iron depletion (n = 251), cellular iron deficiency (n = 251), vitamin A deficiency (VAD, based on serum retinol (SR) values; n = 221), VAD (based on retinol binding protein (RBP) values; n = 251) and riboflavin deficiency (n = 251), stratified by age-group. **B.** Prevalence of *P. falciparum*, soil-transmitted helminth and schistosome infection (n = 375), acute inflammation (n = 251), chronic inflammation (n = 251), storage iron depletion (n = 251), cellular iron deficiency (n = 251), VAD (based on SR values; n = 221), VAD (based on RBP values; n = 251) and riboflavin deficiency (n = 251), stratified by locality. The prevalence of micronutrient deficiency is calculated for participants with complete parasitologic datasets, but without acute inflammation (CRP ≤ 10 mg/L) in the Taabo HDSS, April 2010. CRP, C-reactive protein; AGP, α1-acid glycoprotein; sTfR, soluble transferrin receptor; EGRAC, erythrocyte glutathione reductase activity coefficient.

The overall prevalence of infection with any helminth species was 13% in Taabo Cité, 33% in Ahondo and 41% in Katchénou. In the latter setting, 53% of school-aged children and women were found infected. *S. haematobium* was mainly encountered in Ahondo, where 40% of school-aged children and women had a positive urine filtration result. Of the 102 participants positive for any helminth species, only two presented with a hookworm infection of heavy intensity ($\geq 4,000$ EPG) and five with a heavy *S. haematobium* infection (≥ 50 eggs/10 ml of urine). All other helminth infections were of light or moderate intensity.

The prevalence of acute inflammation (17%), as indicated by elevated CRP, was moderate in all study areas and in all age groups. Interestingly, the prevalence of chronic infection or inflammation, as indicated by high AGP values (37%), was the highest in infants (60%) despite a considerably higher prevalence of *P. falciparum* parasitaemia and helminth infection in school-aged children.

Around 15% of participants from both Taabo Cité and Ahondo showed storage iron depletion, whereas 4% of the study population in Katchénou was concerned by this shortage. Infants constituted the group at highest risk of cellular ID (71%, $\chi^2 = 29.03$; $P < 0.001$) and storage iron depletion (29%; $\chi^2 = 32.15$; $P < 0.001$). Considerably less school-aged children suffered from cellular ID (38%) and only 3% showed reduced storage iron. The respective percentage of young women considered iron deficient and iron depleted was 34% and 12%, respectively. Regardless of the choice of the marker, the repartition of VAD was similar in the three settings. Infants and young school-aged children were the most concerned by VAD. Based on HPLC measures, VAD was found in 31% of all study participants, whereas RBP determined a percentage of 19%. RBP showed a specificity of 94% and a sensitivity of 55% compared to retinol measures done with HPLC and both methods correlated well (Spearman's $\rho = 0.72$). Two-third of the women suffered from riboflavin deficiency with an EGRAC > 1.4 , suggesting clear deficiency and, altogether, more than 50% of the participants showed reduced riboflavin values. Hb phenotypes determined through electrophoresis revealed that 84.3% carried adult Hb (HbAA), 7.1% carried a C allele (HbAC), 7.9% carried a S allele (HbAS) and 0.8% had sickle cell anaemia (HbSS).

3.4.6 Risk factors for anaemia, stratified by age

Table 3.3 summarizes the statistically significant ($P < 0.05$) risk factors for anaemia, as determined by multivariable logistic regression, stratified by age. Locality was included as a random effect in the models. In infants, *P. falciparum* was the only significant risk factor for anaemia (OR = 8.08, 95% CI 1.69-38.63). Cellular ID was a risk factor of anaemia in both school-aged children and young non-pregnant women. Moreover, school-aged children with chronic inflammation were more likely to have anaemia than children without chronic

inflammation (OR = 3.58, 95% CI 1.56-8.25). In young women, riboflavin deficiency was associated with a lower odds of anaemia (OR = 0.29, 95% CI 0.10-0.87).

Table 3.3: Risk factors significantly associated with anaemia in infants, school-aged children and young non pregnant women living in Taabo Cité, Ahondo and Katchénou, in April 2010, as determined with multivariate logistic regression with locality as random effect (n = 306).

Outcome	Group	Risk factor	OR	95% CI	Wald-test P-value
Anaemia ¹	Infants ²	<i>P. falciparum</i> infection	8.1	1.69-38.63	0.009
		Chronic inflammation ⁴	3.6	1.56-8.25	0.003
	Young women ⁵	Cellular iron deficiency	2.3	1.02-5.02	0.045
		Cellular iron deficiency	6.0	1.75-20.59	0.004
		Riboflavin deficiency	0.3	0.10-0.87	0.027
		Occupation: housekeeper	0.2	0.03-0.78	0.038

¹ According to WHO criteria: Hb <12g/dl for non-pregnant women, Hb <11.5g/dl for children, Hb <11g/dl for infants

² The original model included the following explanatory variables: age, sex, cellular iron deficiency, storage iron depletion, riboflavin deficiency, vitamin A deficiency, chronic inflammation, acute inflammation, *P. falciparum*, schistosomiasis and soil-transmitted helminthiasis. Stepwise backward multivariate logistic regression was performed keeping only explanatory variables with p-values <0.2. OR adjusted for chronic inflammation and storage iron depletion (n = 95).

³ The original model included age, sex, school attendance, cellular iron deficiency, storage iron depletion, riboflavin deficiency, vitamin A deficiency, chronic inflammation, acute inflammation, *P. falciparum*, schistosomiasis and soil-transmitted helminthiasis. OR adjusted for soil-transmitted helminthiasis and school attendance (n = 133)

⁴ Defined as AGP >1 g/l

⁵ The original model included age, occupation, chronic inflammation, acute inflammation, *P. falciparum*, soil-transmitted helminthiasis, schistosomiasis, cellular iron deficiency, storage iron depletion, retinol deficiency and riboflavin deficiency. OR adjusted for schistosomiasis and occupation (n = 78).

3.5 Discussion

Data from the current cross-sectional survey on anaemia and underlying causes revealed that 78% of infants and around 50% of school-aged children and non-pregnant women were anaemic in two rural and one urban setting of south-central Côte d'Ivoire. Our findings therefore underscore that anaemia is an important public health issue in Côte d'Ivoire (Staubli-Asobayire et al., 2001). The identification of risk factors for the three specific age classes studied here (i.e., infants aged 6-23 months, children aged 6-8 years and non-pregnant women aged 15-25 years) confirmed the multiple and complex aetiology of anaemia in a typical, primarily rural setting of sub-Saharan Africa (Tolentino and Friedman, 2007). We found specific risk factors for anaemia depending on age, which calls for the development and implementation of an integrated approach targeting these vulnerable groups.

The main limitations of the study presented here are as follows. First, the overall compliance was low; from the 732 people initially invited to participate, less than 50% had complete data records. Particularly young women showed low compliance (28%). Reasons for the low compliance are numerous: the challenge to motivate people to participate, the difficulty to collect urine and stool samples from infants and people's hesitation to provide venous blood, particularly in areas where HIV/AIDS is present. Moreover, the collection of sufficiently large volumes of venous blood that was necessary for the battery of tests employed here proved difficult, particularly for infants. Second, the choice of our study settings was a combination of representativeness and operational feasibility. Since our longitudinal monitoring would last for more than 1 year, with visits once every 3-4 months, the accessibility of the chosen localities throughout the study period was a mandatory criterion. Third, there are some shortcomings regarding the methods used for appraisal of micronutrient deficiencies, such as indirect estimation of the riboflavin status and non-universally recognized cut-off values for sTfR (WHO/UNICEF/UNU, 2001).

Our results indicate that *S. haematobium* and soil-transmitted helminth infections are present in all three study settings, but with low overall prevalence and infection intensities. Hence, compared to previous research and thanks to local control efforts implemented in the Taabo district, major improvements regarding *S. haematobium* endemicity have been achieved (N'Goran et al., 1997; 2001; 2003). Of note, surveys done in farming communities require the collection of urine samples in the early morning, as farmers leave their homes early and they only return in the late afternoon after having worked in the fields. However, *S. haematobium* egg output is highest around mid-day and hence the 'true' prevalence of *S. haematobium* is

likely to have been underestimated (WHO, 1998). With regard to soil-transmitted helminths, hookworm was the predominant species. Since only one stool specimen was subjected to duplicate Kato-Katz thick smear examination, it is conceivable that the reported prevalence of soil-transmitted helminths is a considerable underestimation of the 'true' infection prevalence (Marti and Koella, 1993; Utzinger et al., 2001; Knopp et al., 2008; Knopp et al., 2009). This issue is important, as it might have reduced the likelihood of documenting a significant relationship between helminth infection and anaemia.

A previous study conducted in villages in close proximity to the Taabo HDSS revealed that the administration of anthelmintic drugs (albendazole plus praziquantel) to school-aged children twice with a 3-month interval significantly increased Hb concentration by 2.4 g/l (Rohner et al., 2010). A recent meta-analysis revealed that *S. haematobium* and hookworm infections were significantly associated with anaemia in children < 4 years old and that up to 10% of anaemic cases could be averted by treating helminth infections (Soares Magalhães and Clements, 2011). The current data suggest that helminth infection was not a significant risk factor for anaemia for none of the three age groups investigated. Nevertheless, since albendazole and ivermectin have been administered at the population level after the baseline cross-sectional survey reported here, the results from subsequent surveys will shed new light on the long-term effect of deworming campaigns against anaemia.

The present survey was conducted shortly before the rainy season. Interestingly though, the prevalence of *P. falciparum* infection was slightly higher than what has been reported before for school-aged children in the same area (Rohner et al., 2010). Our data confirm that malaria is still highly endemic in Côte d'Ivoire, perhaps explained by the prolonged socio-political crises (Bonfoh et al., 2011) and the overall low coverage rate of proven interventions, such as LLINs (Noor et al., 2009). Although malaria has a wide range of clinical outcomes, malaria-related anaemia is one of the leading causes of death, particularly in children (Murphy and Breman, 2001). In our study, infection with *P. falciparum* was the only risk factor significantly associated with anaemia in infants. Our data also suggest that anaemia of chronic disease (ACD), also phrased anaemia of inflammation, plays a role in the process of anaemia for children aged 6-8 years. Interestingly, only 14% and 36% of school-aged children had an elevated CRP and AGP, respectively, despite a considerably higher prevalence of *Plasmodium* parasitaemia. This discrepancy may, at least partly, be explained by the generally low parasitaemia found in this age group and indicates that afebrile malaria is not necessarily associated with increased AGP and CRP. These observations were inversed in infants; whilst slightly less than 40% had a positive RDT, 60% showed elevated AGP values. This result may be explained by other illnesses which can increase inflammatory markers in children during their first years of life. Intestinal and urinary tract

infection, respiratory diseases, hepatitis, measles, HIV and small injuries are among factors which affect the young child and could increase inflammatory markers.

The body of evidence pertaining to ID and its major contribution to anaemia is compelling (WHO/UNICEF/UNU, 2001; Stoltzfus, 2003). Clearly, other micronutrients such as vitamin A, B12 and folate are important factors in the pathophysiology of anaemia, although we still lack intervention studies that confirm and quantify these associations (Haider and Bhutta, 2011; Zimmermann et al., 2006; West et al., 2007; Scott, 2007). Our study confirms that iron-deficiency anaemia is prevalent in south-central Côte d'Ivoire (Staubli-Asobayire et al., 2001; Cercamondi et al., 2010). However, our results also emphasize the lack of robust markers for estimating ID in populations where malaria and, more generally, inflammation, are widespread. Both ferritin and sTfR showed a significant correlation with AGP and CRP. sTfR weakly but significantly correlated with CRP (Spearman's $\rho = 0.26$; $P < 0.01$), in contrary to previous studies which showed that the specificity and sensitivity of sTfR in estimating ID remained, even in areas endemic for malaria, unaffected by the acute phase response (Staubli-Asobayire et al., 2001; Beguin 2003).

Although we found the highest prevalence of ID and iron depletion in infants, these parameters were not identified as significant risk factors for anaemia in this age group. Chronic inflammation was the only variable significantly negatively associated with anaemia. It has been shown that iron fortification can prevent anaemia in infants free of malaria (Walter et al., 1993) and a recent meta-analysis showed that iron supplementation is recommended in area where malaria is endemic when regular malaria surveillance and treatment services are provided (Okebe et al., 2011). Of note, sTfR is a marker of cellular iron demand and there are mid-to-moderate forms of ID where anaemia is absent. These forms of ID often lead to anaemia if they remain untreated. This observation is reflected in the school-aged children for whom ID becomes an important risk factor. Iron fortification or supplementation coupled to the distribution of LLINs and IPT of malaria in infants would be effective strategies to prevent the development of ID anaemia in our population. Moreover, although the prevalence of ID was slightly lower in young women, it represents a significant risk factor for this age group, confirming the body of evidence about the burden of ID in women of childbearing age.

Riboflavin deficiency was very common in the study populations. Indeed, 60% of the women, half of the 6- to 8-year-old children and more than 40% of infants showed riboflavin deficiency. These results are not surprising considering the low intake of dairy products in our study population. Although there is only little data on the riboflavin status of populations from sub-Saharan Africa, other studies suggest that this deficiency may be common in this

part of the world (Faber et al., 2001; Abrams et al., 2003; Siekmann et al., 2003; Rohner et al., 2007). Riboflavin supplementation has been shown to improve haematological status alone or in combination with iron in deficient populations (Powers et al., 1983; Powers et al., 2011). Interestingly, our study is the first which showed lower odds of anaemia for young women with riboflavin deficiency. One hypothesis is that riboflavin deficiency protects from malaria and indirectly improves haematological parameters (Kaikai and Thurnham, 1983; Thurnham et al., 1983). Nevertheless, multivariate regression analysis and Wilcoxon rank-sum test revealed that riboflavin deficiency and EGRAC values were neither associated with malaria prevalence nor with *Plasmodium* parasitaemia in women. Moreover, riboflavin deficiency was also significantly negatively associated with anaemia in women free of malaria suggesting another mechanism. It is also worth noting that EGRAC is a method which results rely on the activity of erythrocyte glutathione reductase (EGR). Thus, even in populations where dairy food is not consumed daily, caution is indicated with the interpretation of abnormal EGRACs as indicators of true nutritional riboflavin deficiency. A previous study done in neighbouring villages showed that daily intake of riboflavin was insufficient in children (Rohner et al., 2007), suggesting that at least a part of the low EGRACs observed in our population is due to a true riboflavin deficiency.

We found that one third of children were vitamin A deficient, confirming previous findings from a neighbouring locality (N'Goran et al., 2003). RBP showed a rather low sensitivity (55%) but good specificity (94%) in the detection of VAD. However, the definition of VAD according to RBP or serum retinol did not modify the results of our multivariate logistic regression in school-aged children and young women. Moreover, within the 62 infants for whom vitamin A status was also assessed by HPLC, this parameter was not correlated to Hb. Although vitamin A supplementation has shown a positive effect on haematological status of children in areas free of malaria (Zimmermann et al., 2006), VAD was not a significant risk factor for anaemia in participants free of malaria.

We conclude that although anaemia is multifactorial, our study revealed that there are specific risk factors for specific age groups. Infants are primarily affected by malarial anaemia; older children by anaemia of inflammation and/or ID anaemia. The latter is also the main risk factor of anaemia in young non-pregnant women. Our study moreover brought to light that iron and in riboflavin can have an opposite association with anaemia. Taken together, our observations call for interventions targeting malaria and the large-scale distribution of LLINs to mothers of preschool-aged children should be done without delay. Additionally, dietary improvements must be explored. In view of the importance of anaemia of inflammation in school-aged children, regular deworming and community-led total sanitation and health education to improve sustainability, should be considered. After the current

baseline cross-sectional survey completed in April 2010, interventions have been launched, but there were interruptions due to the post-election crisis in late 2010/early 2011. The results communicated here will serve as a benchmark for longitudinal monitoring, including the impact of armed conflict and war.

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4. Interactions and potential implications of *Plasmodium falciparum*-hookworm coinfection in different age groups in south-central Côte d'Ivoire

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4.1 Abstract

Background: Given the widespread distribution of *Plasmodium* and helminth infections, and similarities of ecological requirements for disease transmission, coinfection is a common phenomenon in sub-Saharan Africa and elsewhere in the tropics. Interactions of *Plasmodium falciparum* and soil-transmitted helminths, including immunological responses and clinical outcomes of the host, need further scientific inquiry. Understanding the complex interactions between these parasitic infections is of public health relevance considering that control measures targeting malaria and helminthiases are going to scale.

Methodology: A cross-sectional survey was carried out in April 2010 in infants, young school-aged children, and young non-pregnant women in south-central Côte d'Ivoire. Stool, urine, and blood samples were collected and subjected to standardized, quality-controlled methods. Soil-transmitted helminth infections were identified and quantified in stool. Finger-prick blood samples were used to determine *Plasmodium* spp. infection, parasitaemia, and haemoglobin concentrations. Iron, vitamin A, riboflavin, and inflammation status were measured in venous blood samples.

Principal Findings: Multivariate regression analysis revealed specific association between infection and demographic, socioeconomic, host inflammatory and nutritional factors. Non-pregnant women infected with *P. falciparum* had significantly lower odds of hookworm infection, whilst a significant positive association was found between both parasitic infections in 6- to 8-year-old children. Coinfected children had lower odds of anaemia and iron deficiency than their counterparts infected with *P. falciparum* alone.

Conclusions/Significance: Our findings suggest that interaction between *P. falciparum* and light-intensity hookworm infections vary with age and, in school-aged children, may benefit the host through preventing iron deficiency anaemia. This observation warrants additional investigation to elucidate the mechanisms and consequences of coinfections, as this information could have important implications when implementing integrated control measures against malaria and helminthiases.

4.2 Author Summary

In sub-Saharan Africa, parasitic worms (helminths) are among the most common chronic infections, malaria among the most deadly, and coinfection is the norm rather than the exception. Infections with hookworm and *Plasmodium* can decrease the level of haemoglobin and are therefore associated with anaemia. Previous studies have investigated the consequences of coinfection in different age groups and settings, but results are conflicting. Indeed, there is no consensus about detrimental or beneficial effects of a coinfection for the host. We designed a cross-sectional study to determine risk factors for anaemia and investigated interactions and discuss potential implications of *P. falciparum* and hookworm coinfection in three groups of people. Overall, 324 individuals were diagnosed for helminths and *Plasmodium* infections, anaemia, subclinical inflammation, and micronutrient deficiencies, and household's socioeconomic status was determined based on an asset-index. We found significant associations between hookworm and *P. falciparum* infections, depending on the age group. Interestingly, 6- to 8-year-old children harbouring a coinfection showed significantly lower odds of anaemia and iron deficiency than children infected with *P. falciparum* alone. This observation warrants follow-up studies, as there are important implications when implementing integrated control measures against malaria and helminthiases.

4.3 Introduction

Recent estimates indicate that approximately 30% of the world's population is still exposed to malaria and that most clinical events attributable to *Plasmodium falciparum* are concentrated in the African region (Snow et al., 2005). Hookworm (*Ancylostoma duodenale* and *Necator americanus*) and other soil-transmitted helminths are also widespread, affecting more than a billion people with an estimated 200 million cases of hookworm infections found in sub-Saharan Africa (de Silva et al., 2003). Given the widespread distribution of *Plasmodium* and soil-transmitted helminth infections, and the similarity of ecological requirements for disease transmission, coinfection is a common phenomenon. For instance, it has been estimated that over a quarter of school-aged children in sub-Saharan Africa, are at risk of *Plasmodium*-hookworm coinfection (Brooker et al., 2006).

The interactions between soil-transmitted helminths and *P. falciparum*, including immunological responses and clinical outcomes of the host, are not well understood (Hartgers and Yazdanbakhsh, 2006). While some studies observed an inverse relationship between helminthiasis and malaria (Murray et al., 1978; Brutus et al., 2007; Kung'u et al., 2009; Melo et al., 2010), other studies suggest that coinfection may be more frequent than expected by chance, and hence exacerbate disease of a single infection (Nacher et al., 2002; Spiegel et al., 2003; Yatich et al., 2009; Pullan et al., 2011). These conflicting patterns have been observed in different age groups for both males and females. Importantly, since chronic helminth infection and *P. falciparum* can lead to anaemia, the risk of anaemia and iron deficiency among coinfecting individuals might be exacerbated (Brooker et al., 2007).

Risk factors and the consequences of single and multiple species parasite infection may vary depending on setting, age, infection intensity, and the host's nutritional status. A deeper understanding of such risk factors has important public health implications, and it is of considerable relevance as control interventions against malaria and helminthiasis are going to scale (Hotez et al., 2007; Murray et al., 2012).

Here, we report results from a baseline cross-sectional survey as part of a 14-month prospective longitudinal surveillance of anaemia in three cohorts (infants aged 6-23 months, children aged 6-8 years, and young women aged 15-25 years) implemented on the site of the recently established Taabo health demographic surveillance system (Taabo HDSS) in south-central Côte d'Ivoire. Emphasis is placed on *P. falciparum* and helminth infections, and micronutrient deficiencies. The specific objectives of the study reported here were (i) to assess the prevalence and intensity of *P. falciparum* and helminth infection, (ii) to evaluate the association between *P. falciparum* and helminth infections in the three population groups,

and (iii) to discuss potential implications of *P. falciparum*-hookworm coinfection in relation to anaemia in young school-aged children.

4.4 Materials and methods

4.4.1 Ethics statement

Ethical approval was granted by the ethics committee of Basel (EKBB, reference no. 252/09) and Côte d'Ivoire (reference no. 1086 MSHP/CNER). Study investigators were covered by liability insurance (GNA Assurance; Abidjan, Côte d'Ivoire, policy no. 30105811010001). Village authorities and participants were informed about the purpose, procedures, and potential risks and benefits of the study. Written informed consent was obtained from study participants and the parents/guardians of children below the age of 15 years. Suspected clinical malaria (i.e., positive rapid diagnostic test (RDT) and tympanic temperature $\geq 38^{\circ}\text{C}$), severe anaemia (i.e., haemoglobin (Hb) < 8 g/dl according to national guidelines of Côte d'Ivoire defining anaemia requiring appropriate intervention), and helminth infections were treated according to national guidelines.

4.4.2 Study area

This study was carried out in the recently established Taabo HDSS, which covers Taabo sub-district located in south-central Côte d'Ivoire (N'Goran et al., 1997). Altitudes of the study area are between 50 and 250 m above sea level. The results presented here stem from the baseline cross-sectional survey of a 14-month prospective longitudinal monitoring, which aims to further the understanding of the aetiology of anaemia, including measures for prevention and control. The Taabo HDSS consists of a small district town (Taabo Cité), 13 main villages, and over 100 hamlets. Approximately 38,500 people are under demographic and health surveillance. The longitudinal monitoring reported here was implemented in three localities: (i) Taabo Cité, the only small town of Taabo HDSS; (ii) Ahondo, one of the 13 main villages, situated in close proximity to Lake Taabo; and (iii) Katchénou, a hamlet (subsequently designated a small village), located 50 km south of Taabo Cité.

4.4.3 Field procedures

Details of the field procedures have been described elsewhere (Righetti et al., 2012). In brief, we designed a prospective longitudinal study pertaining to anaemia and potential nutritional and parasitic risk factors in three age groups, namely (i) infants (aged 6-23 months), (ii) children in early school-age (6-8 years), and (iii) young women (aged 15-25 years). The choice of these three age groups was based on the high vulnerability of infants and women

of childbearing age to the consequences of anaemia, and the important exposure, yet non-immunity, of young school-aged children to parasitic infections. Based on an estimated proportion of anaemia of 60% in infants and school-aged children and 40% in non-pregnant women, a drop-out rate of 20% and a confidence level of 95%, we intended to sample 137 individuals for each of the three age-groups from the readily available Taabo HDSS database, to produce an accurate estimation of the prevalence of anaemia with a 9% error margin. Since the participation rate during the initial sampling phase was lower than expected, all individuals meeting our age (and sex) requirements from Ahondo and Katchénou were invited to participate. In Taabo Cité, a stepwise random sampling was employed and 120 infants, 90 children of early school-age, and 90 young non-pregnant women were selected from the Taabo HDSS database.

Overall, 732 individuals were invited to participate. Venous and finger-prick blood, stool, and urine samples were collected and subjected to standardized, quality-controlled methods to diagnose and quantify *Plasmodium* and helminth infections, and determine the participants' micronutrient status.

4.4.4 Laboratory Analyses

The presence of *P. falciparum* was determined using finger-prick blood and an RDT (ICT ML01 malaria Pf kit; ICT Diagnostics, Cape Town, South Africa). The determination of *Plasmodium* species was done with a thin blood film, and parasitaemia assessed with a thick blood film. Hb quantification was done with a portable HemoCue Hb 301 device (HemoCue AB; Ängelholm, Sweden).

Duplicate Kato-Katz thick smears (using 41.7 mg templates) were prepared from each stool sample (Katz et al., 1972) and examined under a microscope for the presence of soil-transmitted helminth (*Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*) and *Schistosoma mansoni* eggs. The number of eggs was counted and recorded for each species separately. For each individual, the egg counts of both Kato-Katz thick smears were added and multiplied by a factor 12 to obtain a standardized measure of infection intensity (i.e., eggs per gram of stool (EPG)). Urine samples were subjected to a filtration method (10 ml) (WHO 1991) and slides quantitatively examined under a microscope for *S. haematobium* eggs.

For quality control, 10% of the Kato-Katz thick smears and urine filters were reexamined by a senior technician. In case of conflicting results, the slides were reexamined and results discussed with the technicians until consensus was reached.

Venous blood samples were centrifuged, aliquoted, and kept at -20°C before transfer in an ice-cold box to the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (Abidjan, Côte d'Ivoire) and then to ETH Zurich (Zurich, Switzerland). Riboflavin was measured by the erythrocyte glutathione reductase activity coefficient (EGRAC) assay, using the method of Dror et al. (Dror et al., 1994) with some modifications validated in our laboratory. A cut-off value >1.4 was used to define riboflavin deficiency (Sauberlich, 1999). Serum ferritin, soluble transferrin receptor (sTfR), retinol binding protein (RBP), α 1-acid glycoprotein (AGP), and C-reactive protein (CRP) were measured with a sandwich enzyme-linked immunosorbant assay (ELISA) that has been described elsewhere (Erhardt et al., 2004).

4.4.5 Statistical analysis

Data were entered twice using Microsoft Access version 10.0 (2007 Microsoft Corporation) and the two datasets compared with EpiInfo version 3.4.1 (Centers for Disease Control and Prevention; Atlanta, GA, USA). All statistical analyses were performed with STATA version 10 (StataCorp.; College Station, TX, USA).

Anaemia was defined as Hb <11.0 g/dl for infants, <11.5 g/dl for children aged 6-8 years, and <12.0 g/dl for non-pregnant women, according to guidelines put forward by the World Health Organization (WHO) (WHO/UNICEF/UNU, 2001). Storage iron depletion was defined as ferritin <12 μ g/l for infants without inflammation, and <15 μ g/l for school-aged children and women without inflammation. For participants with AGP >1 g/l or CRP >10 mg/l, storage iron depletion was defined as ferritin <30 μ g/l (WHO/UNICEF/UNU, 2001) Cellular iron deficiency was defined as sTfR >8.5 mg/l (Cook et al., 1993). Vitamin A deficiency was defined as RBP <0.825 μ mol/l (Gorstein et al., 2008).

Only those individuals who had complete datasets (i.e., demographic, parasitological, and micronutrient data) were included for detailed statistical analyses ($n = 324$). For calculating household socioeconomic status, an asset-based index was constructed for each household of the participants with complete data records, according to a method described by Filmer and Pritchett (Filmer and Pritchett, 2001). In brief, data on household assets (e.g., radio), housing characteristics (e.g., wall type), and number of people per room were obtained from the Taabo HDSS database. The binary data of these variables were weighted using principal component analysis (PCA), and the households were subsequently divided into five socioeconomic groups (wealth quintiles); namely (i) most poor, (ii) very poor, (iii) poor, (iv) less poor, and (v) least poor. The procedure is further explained and illustrated in technical notes provided by the Health, Nutrition, and Population (HNP) Poverty Thematic Group of the World Bank (Gwatkin et al., 2000) and elsewhere (O'Donnell et al., 2008).

Crude odds ratios (ORs) were calculated for variables potentially associated with *P. falciparum* (defined as a positive RDT or the presence of *Plasmodia* on blood films) and hookworm infection. To assess independent predictors of *P. falciparum* and hookworm infection, a multivariate logistic regression model was fitted and standard error adjusted for potential clustering within household. An independent model was computed for each age group.

Student's *t*-test and Wilcoxon rank-sum test were used for comparison of means and ranks, respectively. Categorical data were compared using χ^2 test or Fisher's exact test, as appropriate.

4.5 Results

4.5.1 Attrition analysis

From the 732 individuals invited to participate in our prospective longitudinal study, 407 (55.6%) provided written informed consent and 324 (44.3%) had complete data records (i.e., anthropometric, hematologic, parasitic, and micronutrient data). The sex ratio was balanced among infants and school-aged children who were lost to follow-up and those with complete data. There was no difference in mean age in infants, school-aged children and women who were lost to follow-up and those with complete data. There was a significant difference in participation rate across study settings (Taabo Cité: 44%, Ahondo: 34%, Katchénou: 64%; $p < 0.001$).

4.5.2 Prevalence of *P. falciparum* infection and parasitaemia

According to the RDT and the blood film examinations, 58.0% (95% confidence interval (CI) 52.4-63.5%) of the study participants were found infected with *P. falciparum*. Children aged 6-8 years showed the highest prevalence (78.2%). The respective prevalence in infants and non-pregnant women were 45.3% and 36.6% (**Table 4.1**). Among those found infected with *P. falciparum*, 30.2% of infants, 9.6% of school-aged children, and 3.3% of young non-pregnant women harboured $>5,000$ parasites/ μ l of blood, respectively. Four infants aged between 7 and 14 months and one 8-year-old child presented with clinical malaria.

4.5.3 Prevalence and intensity of helminth infection

Overall, 72 of the 324 participants (22.2%) with complete data records had an infection with soil-transmitted helminths. Hookworm was the predominant species (21.6%), whereas only

eight (2.5%) and four (1.2%) individuals were infected with *T. trichiura* and *A. lumbricoides*, respectively.

There were only three cases (3.2%) of soil-transmitted helminth infection in infants, whereas a prevalence of 29.9% and 30.5% was found in young school-aged children and non-pregnant women, respectively (Table 4.1). Among children aged 6-8 years, infection with *A. lumbricoides* and *T. trichiura* was found in three and six individuals, whereas one case of *T. trichiura* was found in non-pregnant women and one case of each *T. trichiura* and *A. lumbricoides* was observed among infants.

S. haematobium infection was found in 35 individuals (10.8%) with the highest prevalence observed in non-pregnant women (22.0%). The respective prevalence in school-aged children and infants was 10.2% and 2.1%.

Helminth infections were primarily of low intensity, regardless of the age groups. Only two participants, children aged 6 and 8 years, presented with heavy hookworm infection (≥ 4000 EPG).

Table 4.1: Prevalence and intensity of infection.

	Infants (<i>n</i> = 95)	School-aged children (<i>n</i> = 147)	Women (<i>n</i> = 82)	p-value*
Soil-transmitted helminth infection				
Prevalence (total), % (95% CI)	3.2 (0.7-9.0)	29.9 (22.7-38.0)	30.5 (20.8-41.6)	<0.001
Hookworms prevalence, % (95% CI)	1.1 (0.0-5.7)	29.9 (22.7-38.0)	30.5 (20.8-41.6)	<0.001
<i>Ascaris lumbricoides</i> prevalence, % (95% CI)	1.1 (0.0-5.7)	2.0 (0.4-5.8)	0	0.695
<i>Trichuris trichiura</i> prevalence, % (95% CI)	1.1 (0.0-5.7)	4.1 (1.5-8.7)	1.2 (0.0-6.6)	0.336
Median hookworms egg count (for positive cases), EPG	252	204	72	
Mean hookworms egg count (for positive cases), EPG	252	752	203	
S. haematobium infection				
Prevalence, % (95% CI)	2.1 (0.3-7.4)	10.2 (5.8-16.3)	22.0 (13.6-32.5)	<0.001
Median number of eggs of <i>S. haematobium</i> /10 ml urine (for positive cases)	1	16	11	
Mean number of eggs of <i>S. haematobium</i> /10 ml urine (for positive cases)	1	38	36	
P. falciparum infection				
Prevalence, % (95% CI)	45.3 (35.0-55.8)	78.2 (70.7-84.6)	36.6 (26.2-48.0)	<0.001
Median number of parasites/ul blood (for positive cases)	2440	740	192	
Mean number of parasites/ul blood (for positive cases)	11461	4163	1081	
Coinfection				
Prevalence of <i>P. falciparum</i> -hookworms coinfection	1.1 (0.0-5.7)	27.9 (20.8-35.9)	4.9 (1.3-12.0)	<0.001
Prevalence of <i>P. falciparum</i> -schistosome coinfection	1.1 (0.0-5.7)	8.8 (4.8-14.6)	8.5 (3.5-16.8)	0.004

Prevalence, median and arithmetic means as determined from stool, urine and finger-prick blood samples collected from 324 individuals in April 2010, in south-central Côte d'Ivoire. P-value based on χ^2 or Fisher's exact test between groups

4.5.4 Parameters associated with *P. falciparum* and helminth infections

Tables 4.2 and 4.3 present ORs between demographic, socioeconomic, inflammatory, and micronutrient parameters and *P. falciparum* and hookworm infection, respectively, as determined by univariate and multivariate logistic regression analyses. Our multivariate regression model revealed that vitamin A deficiency (OR = 10.26, 95% CI 1.89-55.54), inflammation (OR = 4.74, 95% CI 1.12-20.04), and setting (OR (Katchénou) = 30.20, 95% CI 2.60-350.65) were significantly associated with *P. falciparum* infection in infants. Vitamin A deficiency (OR = 10.79, 95% CI 2.68-43.49), cellular iron deficiency (OR = 5.38, 95% CI 1.56-18.56), inflammation (OR = 5.36, 95% CI 1.78-16.13), and hookworm infection (OR = 7.47, 95% CI 1.84-30.32) were significantly associated with higher odds of *P. falciparum* infection in children aged 6-8 years. Moreover, the odds of *P. falciparum* infection among school-aged children were significantly lower for older children (OR (8 years) = 0.25, 95% CI 0.07-0.92). For young non-pregnant women, a concurrent hookworm infection was significantly associated with lower odds of *P. falciparum* infection (OR = 0.14, 95% CI 0.03-0.60). Non-pregnant women had a lower odds of *P. falciparum* infection if they belonged to the least poor quintile (OR = 0.06, 95% CI 0.00-0.86).

Following multivariate analysis with hookworm infection as outcome, age 8 years (OR = 4.56, 95% CI 1.11-18.69), infection with *P. falciparum* (OR = 7.29, 95% CI 1.36-39.23), and setting (OR (Katchénou) = 7.85, 95% CI 1.05-58.82) were significantly positively associated with hookworm infection in school-aged children. Intermediate socioeconomic status was associated with decreased odds of hookworm infection among these children. Furthermore, non-pregnant women infected with *P. falciparum* had lower odds of hookworm infection (OR = 0.16, 95% CI 0.05-0.55). Multivariate logistic regression showed that setting (Ahondo) was the only variable significantly associated with schistosome infection in children and women.

Table 4.2: Variables associated with *P. falciparum* infection.

Variable	Infants (<i>n</i> = 95)			School-aged children (<i>n</i> = 147)			Women (<i>n</i> = 82)		
	Crude OR	Adjusted OR (95% CI)	<i>P</i> value	Crude OR	Adjusted OR (95% CI)	<i>P</i> value	Crude OR	Adjusted OR (95% CI)	<i>P</i> value
Sex									
Male	1.00			1.00					
Female	1.08	3.39 (0.71-16.06)	0.125	0.56	0.53 (0.15, 1.86)	0.128	N/A		
Age class									
Younger	1.00			1.00			1.00		
Middle	2.10	0.92 (0.18-4.69)	0.917	1.33	1.04 (0.20, 5.33)	0.961	1.31	0.56 (0.18, 1.75)	0.318
Older	2.33	2.10 (0.48-9.19)	0.323	0.49	0.25 (0.07, 0.92)	0.036	N/A		
Setting									
Taabo Cité	1.00			1.00			1.00		
Ahondo	1.13	1.31 (0.35-4.85)	0.686	6.17	7.09 (1.02, 49.18)	0.161	1.90	0.52 (0.11, 2.51)	0.417
Katchénou	12.40	30.20 (2.60-350.65)	0.006	7	3.71 (0.16, 84.87)	0.959	4.46	1.21 (0.11, 12.75)	0.876
Socio-economic status									
Most poor	1.00			1.00			1.00		
Very poor	0.29	0.39 (0.06-2.54)	0.323	1.40	4.63 (0.54, 39.78)	0.163	0.39	0.20 (0.03, 1.35)	0.099
Poor	0.07	1.12 (0.05-25.09)	0.943	0.40	2.83 (0.12, 64.27)	0.513	0.18	0.13 (0.01, 1.67)	0.117
Less poor	0.08	1.47 (0.06-33.61)	0.808	0.41	6.56 (0.25, 172.93)	0.260	0.44	0.36 (0.04, 3.64)	0.385
Least poor	0.04	0.17 (0.01-5.30)	0.316	0.08	0.37 (0.01, 10.56)	0.560	0.10	0.06 (0.00, 0.86)	0.039
Hookworm infection	N/A			5.36	7.47 (1.84, 30.32)	0.005	0.23	0.14 (0.03, 0.60)	0.009
Schistosome infection	N/A			2.08	0.57 (0.06, 5.71)	0.632	1.01	2.42 (0.59, 9.94)	0.222
Cellular iron deficiency	2.52	4.15 (0.93-18.46)	0.061	2.66	5.38 (1.56, 18.56)	0.008	1.13	2.20 (0.50, 9.73)	0.299
Riboflavin deficiency	1.08	0.65 (0.21-2.00)	0.451	0.61	0.59 (0.14, 2.52)	0.474	0.61	0.70 (0.22, 2.23)	0.551
Vitamin A deficiency	3.90	10.26 (1.89-55.54)	0.007	4.03	10.79 (2.68, 43.49)	0.001	N/A		
Inflammation (AGP)	7.59	4.74 (1.12-20.04)	0.034	3.10	5.36 (1.78, 16.13)	0.003	3.27	2.70 (0.22, 2.54)	0.642

Univariate and multivariate logistic regressions were used to calculate the association between *P. falciparum* infection as outcome and demographic, social-ecological, parasitic, nutritional and inflammatory explanatory variables. Adjusted odds ratios (OR) are reported with their 95% confidence intervals. Stool, urine and blood samples were collected from 324 individuals, in April 2010, in south-central Côte d'Ivoire. Significant associations are in bold.

Table 4.3: Variables associated with hookworm infection.

Variable	School-aged children (odds ratio; <i>n</i> = 147)			Women (odds ratio; <i>n</i> = 82)		
	Crude OR	Adjusted OR (95% CI)	<i>P</i> value	Crude OR	Adjusted OR (95% CI)	<i>P</i> value
Sex						
Male	1.00					
Female	0.53	0.50 (0.17, 1.48)	0.209	N/A		
Age class						
Younger	1.00			1.00		
Middle	0.82	0.87 (0.18, 4.09)	0.859	0.77	0.87 (0.26, 2.87)	0.728
Older	2.12	4.56 (1.11, 18.69)	0.035	N/A		
Setting						
Taabo Cité	1.00			1.00		
Ahondo	5.34	4.82 (0.80, 28.97)	0.086	0.84	0.28 (0.04, 1.94)	0.200
Katchénou	43.43	7.85 (1.05, 58.82)	0.045	0.92	0.99 (0.08, 12.29)	0.991
Socio-economic status						
Most poor	1.00			1.00		
Very poor	0.23	0.23 (0.04, 1.23)	0.085	0.54	0.48 (0.07, 3.12)	0.443
Poor	0.07	0.07 (0.01, 0.84)	0.036	2.53	2.88 (0.23, 36.46)	0.414
Less poor	0.02	0.02 (0.00, 0.24)	0.003	0.59	0.64 (0.05, 8.65)	0.735
Least poor	0.13	0.13 (0.01, 2.36)	0.168	0.87	0.41 (0.02, 8.28)	0.564
<i>P. falciparum</i> infection	5.36	7.29 (1.36, 39.23)	0.021	0.23	0.16 (0.05, 0.55)	0.004
Schistosome infection	1.07	1.39 (0.33, 5.85)	0.655	1.97	2.58 (0.63, 10.50)	0.187
Iron deficiency (sTfR)	0.46	0.32 (0.10, 1.03)	0.055	0.78	0.80 (0.23, 2.85)	0.735
Riboflavin deficiency	1.24	2.42 (0.71, 8.25)	0.157	0.59	0.49 (0.16, 1.44)	0.194
Vitamin A deficiency	2.47	1.42 (0.37, 5.41)	0.612	N/A		
Inflammation (AGP)	0.74	0.90 (0.27, 2.96)	0.863	0.74	1.48 (0.26, 8.37)	0.657

Univariate and multivariate logistic regressions were used to calculate the association between hookworm infection as outcome and demographic, social-ecological, parasitic, nutritional and inflammatory explanatory variables. Adjusted odds ratios (OR) are reported with their 95% confidence intervals. Stool, urine and blood samples were collected from 324 individuals, in April 2010, in south-central Côte d'Ivoire. Significant associations are in bold.

4.5.5 Prevalence and implications of *Plasmodium*-hookworm coinfection

As shown in Table 4.1, *P. falciparum*-helminth coinfection was most prevalent among children aged 6-8 years. The prevalence of a concurrent infection with *Plasmodium* and hookworm was 27.9% in school-aged children, whilst the respective prevalence in young non-pregnant women and infants was 4.9% and 1.1%. The prevalence of *P. falciparum*-*Schistosoma* coinfection was low in all age groups.

Considering the overall low prevalence of *P. falciparum*-*Schistosoma* coinfection and *P. falciparum*-hookworm coinfection in infants and women, further investigations focused on *P. falciparum*-hookworm coinfection in the school-aged children. Multivariate regression analysis revealed that sex (OR (female) = 0.33, 95% CI 0.12-0.94) and setting (OR (Ahondo) = 7.53, 95% CI 1.22-46.52; OR (Katchénou) = 11.98, 95% CI 1.64-87.20), was significantly associated with *P. falciparum*-hookworm coinfection among school-aged children (compared to no infection or single species infection). Other demographic, parasitic, and micronutrient parameters were not significantly associated with coinfection status.

Children coinfecting with hookworm and *P. falciparum* had significantly higher concentrations of Hb (Wilcoxon's rank-sum test, $p = 0.038$ ($n = 115$)) and lower sTfR concentrations (Wilcoxon's rank-sum test, $p = 0.010$ ($n = 115$)) than children infected with *P. falciparum* alone. Adjusting for socioeconomic status, sex, age, *P. falciparum* parasitaemia, stunting, inflammation status, and setting revealed that children with a coinfection had significantly lower odds of cellular iron deficiency (OR = 0.17, 95% CI 0.04-0.70) and anaemia (OR = 0.23, 95% CI 0.06-0.83) than their counterparts harbouring a single infection with *P. falciparum*. There was an interaction between age and infection status. **Table 4.4** shows the haematological and inflammation data for school-aged children, stratified by 1-year age increments. Children aged 8 years with *P. falciparum*-hookworm coinfection had significantly higher Hb concentration and lower median values of sTfR, sTfR/log ferritin ratio, and AGP than those infected with *P. falciparum* alone. There was a similar trend for Hb, sTfR, and sTfR/log ferritin in children aged 7 years.

Table 4.4: Implications of *P. falciparum*-hookworm coinfection among school-aged children, stratified by age.

	6-year-old (n = 45)		
	<i>Pf</i> infection	Coinfection	<i>P</i> value
Mean haemoglobin, g/dl (standard deviation)	11.3 (1.0)	11.0 (1.5)	0.739
Geometric mean <i>P. falciparum</i> parasitaemia (95% CI)	1141.3 (495.6, 2626.4)	138.6 (23.2, 840.7)	0.013
Median sTfR, mg/l (interquartile range)	8.1 (6.7, 9.5)	8.7 (6.9, 10.9)	0.987
Median serum ferritin, µg/l (interquartile range)	75.0 (57.2, 140.1)	62.9 (55.1, 85.8)	0.485
Median sTfR/log ferritin (interquartile range)	4.4 (3.4, 5.1)	4.6 (3.8, 5.0)	0.79
Median AGP, g/l (interquartile range)	1.0 (0.8, 1.2)	1.0 (0.7, 1.1)	0.361
Median CRP, mg/l (interquartile range)	3.2 (0.8, 13.6)	1.21 (0.6, 2.2)	0.104
	7-year-old (n = 43)		
	<i>Pf</i> infection	Coinfection	<i>P</i> value
Mean haemoglobin, g/dl (standard deviation)	11.4 (1.0)	11.9 (1.0)	0.182
Geometric mean <i>P. falciparum</i> parasitaemia (95% CI)	490.9 (214.4, 1122.1)	678.8 (227.0, 2017.3)	0.915
Median sTfR, mg/l (interquartile range)	9.0 (7.0, 11.4)	6.7 (5.8, 9.7)	0.107
Median serum ferritin, µg/l (interquartile range)	61.1 (39.5, 108.2)	73.1 (63.3, 94.0)	0.229
Median sTfR/log ferritin (interquartile range)	5.1 (3.6, 6.4)	4.0 (3.1, 4.6)	0.047
Median AGP, g/l (interquartile range)	0.9 (0.7, 1.0)	0.9 (0.9, 1.0)	0.366
Median CRP, mg/l (interquartile range)	1.4 (0.6, 3.4)	2.6 (0.8, 5.7)	0.436
	8-year-old (n = 59)		
	<i>Pf</i> infection	Coinfection	<i>P</i> value
Mean haemoglobin, g/dl (standard deviation)	11.0 (1.1)	11.9 (1.0)	0.009
Geometric mean <i>P. falciparum</i> parasitaemia (95% CI)	280.5 (61.2, 1272.7)	825.5 (480.5, 1417.8)	0.457
Median sTfR, mg/l (interquartile range)	9.2 (8.0, 11.0)	7.6 (6.5, 8.3)	0.007
Median serum ferritin, µg/l (interquartile range)	62.2 (53.7, 116.3)	66.8 (54.1, 81.5)	0.715
Median sTfR/log ferritin (interquartile range)	5.0 (4.3, 5.8)	3.9 (3.5, 5.1)	0.035
Median AGP, g/l (interquartile range)	1.0 (0.9, 1.1)	0.8 (0.7, 1.0)	0.02
Median CRP, mg/l (interquartile range)	1.8 (0.9, 8.1)	3.3 (0.5, 4.2)	0.794

Arithmetic, geometric and median values were derived from blood samples collected from 147 school-aged children, in April 2010, in south-central Côte d'Ivoire. Children infected by *P. falciparum* alone are compared with children matched for age coinfecting by *P. falciparum* and hookworm. Unpaired bilateral t-test was performed to compare means of haemoglobin concentration between groups. Wilcoxon rank-sum test was applied to compare *P. falciparum* parasitaemia, sTfR, ferritin, ratio sTfR/log ferritin, AGP and CRP values between groups. Pf: *P. falciparum*; sTfR: soluble transferrin receptor; AGP: α 1-acid glycoprotein; CRP: C-reactive protein.

4.6 Discussion

Our data derived from a baseline cross-sectional survey among three age groups in south-central Côte d'Ivoire confirm that *P. falciparum* and helminths co-exist with school-aged children at highest risk of coinfection (N'Goran et al., 2003; Becker et al., 2011). We found significant associations between infection status and demographic parameter (age), social-ecological systems (setting and socioeconomic status), and host inflammatory and micronutrient status. Interestingly, young non-pregnant women infected with *P. falciparum* showed significantly lower odds of concurrent hookworm infection, whilst there was a significant positive association between both infections in children aged 6-8 years. Children coinfecting with *P. falciparum* and hookworm had lower odds of anaemia and cellular iron deficiency than their counterparts infected with *P. falciparum* alone. Our findings therefore underscore that the interactions between *P. falciparum* and hookworm are complex and may benefit the host in some circumstances.

4.6.1 Demographic variables

The finding that, in school-aged children, boys had significantly higher odds of coinfection might reflect different recreational exposures between genders. Hence, we cannot generalize our findings about parasites interactions in young women (studied here) to young men (not studied) as the two groups might be exposed differently to parasitic infection (Zuk and McKean, 1996). In school-aged children, growing older was associated with lower odds of *P. falciparum* infection, and the infant-group showed highest *P. falciparum* parasitaemia. Moreover, whilst *P. falciparum* infection was associated with inflammation in infants and school-aged children, there was no significant relationship between both variables in young non-pregnant women.

These observations most likely reflect the clinical and parasitological semi-immunity that individuals living in malaria-endemic areas built up and which protect them from high parasitaemia, clinical malaria, and facilitates the clearance of *Plasmodium* (Doolan et al., 2009). The observation that children aged 8 years had higher odds of hookworm infection compared with their 6-year-old counterparts might be explained by a longer probability of being infected with parasitic worms. Highest helminth infection prevalence is indeed often observed among older children, adolescents, and young adults (Hotez et al., 2006).

4.6.2 Social-Ecological Parameters

Our findings that children at early school-age who live in remote rural areas (i.e., in this study, Katchénou) are at higher risk of hookworm infection and *P. falciparum*-hookworm

coinfection might be explained by the particularly poor hygiene status and suitable ecological conditions for the parasites life-cycles as well as a difficult access to health care facilities for individuals living in this setting. Indeed, at the time of our study, none of the households in Katchénou had sanitation and all of them obtained drinking water from a community pump. The nearest health centre was situated in Sokrogbo, a village located approximately 5 km from Katchénou with difficult access particularly during the rainy season. The observation that remote rural setting (i.e., Katchénou) was significantly associated with *P. falciparum* infection in infants but not in school-aged children might be explained by the high malaria transmission in this setting, increasing the probability that young infants become infected. The high prevalence of *P. falciparum* in all the three study groups emphasizes the importance of implementing prevention and control measures against malaria (WHO, 2011c). Among infants aged 6-23 months, 45% were infected with *P. falciparum* and the parasitaemia level in this age group was higher than in children aged 6-8 years and in non-pregnant women aged 15-25 years. Hence, emphasis should be placed on protecting infants from mosquito bites and on effective management of malaria cases during childhood, most importantly in remote areas where prompt access to quality health care remains a formidable challenge.

4.6.3 Micronutrient Status

With regard to micronutrients, it is not surprising that vitamin A deficiency appeared to be associated with *P. falciparum* infection in infants and school-aged children. Indeed, we have previously shown that *P. falciparum* infection and vitamin A deficiency are prevalent among infants and school-aged children living in our study area (Righetti et al., 2012). Furthermore, although WHO does not recommend vitamin A supplementation before 6 months of age (WHO, 1997; WHO, 2011a; WHO, 2011b), several studies have shown the role and the importance of vitamin A in the pathology of malaria (Shankar et al., 1999; Serghides and Kain, 2002) and, more generally, in the development of the immunological system during childhood (West et al., 1991; Ross, 1993; Jones et al., 2003). It has also been observed that vitamin A utilization may increase during a clinical malaria episode (Galan et al., 1990; Thurnham and Singkamani, 1991), suggesting a vicious cycle between deficiency in vitamin A and *Plasmodium* infection. The cross-sectional nature of our study, however, does not allow drawing causal inference. There are several hypotheses for the significant association we found between iron deficiency and *P. falciparum* infection in infants, based on the haematological and inflammatory consequences of *Plasmodium* infection. On the one hand, erythropoiesis rate might indirectly be increased in response to *Plasmodium* infection, to compensate for the loss of erythrocytes through haemolysis and splenic clearance of infected and uninfected erythrocytes. This increased erythropoiesis might, in turn, be

translated to increased sTfR concentrations (Mockenhaupt et al., 1999; Menendez et al., 2001). On the other hand, the effect of chronic or acute inflammation on sTfR concentrations is still being debated. Whilst several studies have suggested that inflammation inhibits erythropoiesis, others have reported higher sTfR concentrations in individuals with inflammation (Weiss and Goodnough, 2005; Grant et al., 2011). This latest observation might be explained by a secondary haematological response to hepcidin-mediated iron sequestration during inflammatory states.

4.6.4 Interactions and Potential Implications of *Plasmodium*-hookworm Coinfection

Interestingly, our results suggest that the negative association observed between *P. falciparum* and hookworm infection in young non-pregnant women was independent from the social-ecological context. Indeed, multivariate regression analysis showed that this association was independent from age, socioeconomic status, and type of setting of the participating women. We did not find any significant relationship between Hb concentrations and hookworm infection intensity, but it should be kept in mind that our survey was conducted in a setting where helminth infection were primarily of light intensity, probably preventing an important intestinal blood loss in infected subjects (Stoltzfus et al., 1996). One hypothesis for the observed negative association between *P. falciparum* and hookworm infection in women is based on the distinct immunological regulations stimulated by the two parasitic infections (Hartgers and Yazdanbakhsh, 2006). Indeed, helminths are known to activate the immune system with a strong polarization toward T helper 2 (Th2) responses. Furthermore, helminths are often able to survive many years in the host through the induction of immunoregulatory mechanism resulting in an anti-inflammatory environment (Maizels and Yazdanbakhsh, 2003; Taylor et al., 2005). The immune responses to *Plasmodium* are more complex and depend on the species and the stage of infection. The host response toward early infection is rather based on Th1 activation and the production of pro-inflammatory cytokines (Choudhury et al., 2000). These non-specific reactions switch in later stage of *Plasmodium* infection to a Th2 cytokines profile, leading to the production of specific antibodies (Langhorne et al., 1998). Hence, one may suggest that women infected with one parasite species have a more efficient immunological system than women free of infection, leading to a more rapid clearance of a concurrent parasitic infection. Of note, many studies have reported a beneficial effect of helminth infection on malaria (Murray et al., 1978; Brutus et al., 2007; Lyke et al., 2012). An alternative hypothesis is that a specific parasitic infection may deprive a concurrent infection of iron or another nutrient necessary for parasite growth, leading to the elimination of the latter acquired infection. In our cohort, however, women infected with hookworm or *P. falciparum* singly, did not have lower concentrations of

serum ferritin or sTfR than their non-infected counterparts. The observation that children aged 6-8 years coinfecting with *P. falciparum* and hookworm had lower odds of cellular iron deficiency and anaemia, compared to children infected with *P. falciparum* alone, adds to the current debate about *Plasmodium*-helminth coinfection outcomes. A protective effect on anaemia has been observed in early school-aged children concurrently infected by *P. vivax* and soil-transmitted helminths (Melo et al., 2010). The observation that 7- and 8-year-old children infected with *P. falciparum* alone had lower Hb and higher sTfR concentrations compared with children coinfecting with hookworm, whilst serum ferritin concentrations were normal in both groups, suggests that mono-infected children might be more prone to anaemia due to tissue iron deficiency, potentially associated with inflammation (Weiss and Goodnough 2005). Of note, AGP and *P. falciparum* parasitaemia were significantly correlated in children infected with *P. falciparum* alone ($n = 67$; Spearman's $\rho: 0.27$; $p = 0.025$) and this association was not significant for children coinfecting with hookworm ($n = 39$; Spearman's $\rho: 0.04$; $p = 0.807$). Moreover, AGP concentrations were significantly higher in 8-year-old coinfecting children than in children matched for age infected with *P. falciparum* only, indicating that inflammation might indeed be less important in coinfecting children as compared with children infected with *P. falciparum* singly. The measure of hepcidin and inflammatory cytokines and implementation of an anthelmintic drug intervention trial would shed new light on this hypothesis.

In conclusion, our findings emphasize that *P. falciparum* and hookworm infections are associated with demographic, social-ecological, and host inflammatory and micronutrient factors. Coinfection outcomes are complex and might depend on the age and the immune system of the host. New research is needed both in the laboratory and in the field to deepen our understanding of the mechanisms and public health implications of *P. falciparum*-hookworm coinfection. Our observations that coinfection with *P. falciparum* and hookworm are particularly prone in a specific age group (i.e., school-aged children) calls for concerted action in this group. Finally, the finding that light hookworm infection may prevent anaemia in children coinfecting with *P. falciparum* should be considered when implementing integrated prevention and control measures targeting helminthiases and malaria, and call for a surveillance-response approach, so that specific interventions do not harm existing adaptive immune defence mechanisms which might exacerbate morbidity.

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5. Dynamics of anaemia in relation to parasitic infections, micronutrient status, and increasing age in south-central Côte d'Ivoire

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5.1 Abstract

Background. Parasitic diseases (e.g., malaria and helminthiases) exert enormous burdens on public health and social wellbeing. Moreover, parasitic infections are important causes of anaemia in tropical Africa, exacerbated by lack of diversified diet, inflammatory and genetic diseases. There is a paucity of longitudinal studies monitoring the dynamics of anaemia in relation to the aforementioned parameters.

Methods. We designed a 14-month prospective longitudinal study in 3 cohorts (i.e., infants aged 6-23 months, children aged 6-8 years, and women aged 15-25 years), in the Taabo health demographic surveillance system located in south-central Côte d'Ivoire. Parasitological, haematological, and micronutrient data were obtained from repeated cross-sectional surveys, utilizing standardized, quality-controlled methods.

Results. We found that young age, *Plasmodium* and *Schistosoma* infections, cellular iron deficiency and stunting were significantly negatively associated with haemoglobin concentration. Moreover, iron status biomarkers (ie, ferritin and soluble transferrin receptor) were significantly associated with inflammatory parameters.

Conclusions. Based on our results, effective prevention and control measures that target parasitic diseases and iron deficiency are needed. These measures might include the distribution of long-lasting insecticidal nets, intermittent preventive treatment for malaria, regular anthelmintic drug administration, and improved intake of bioavailable iron, coupled with health and nutritional education and improved hygiene, water, and sanitation.

5.2 Introduction

Parasitic diseases, such as malaria and helminthiases, drain the social and economic development of a country and exert an enormous burden on public health and social wellbeing (Sachs and Malaney, 2002; Hotez et al., 2008; Utzinger et al., 2009). Malaria and helminthiases are important causes of morbidity and mortality, particularly in rural communities that lack access to clean water, sanitation, hygiene, and health systems (Bartram and Cairncross, 2010). A common feature shared by malaria and helminthiases is their association with anaemia. Moreover, the lack of a diversified diet with an adequate intake of bioavailable iron, inflammatory diseases, and haemoglobinopathies also significantly contribute to the high burden of anaemia in the tropics (Crawley, 2004; Tolentino and Friedman, 2007). Anaemia in newborns may also be the consequence of poor iron status of the mother prior to or during pregnancy although this relation still lacks evidence (Rasmussen, 2001).

The aetiology of anaemia is multifactorial, and hence there are different preventive and curative measures for its control. For example, iron fortification or supplementation, sleeping under long-lasting insecticidal nets (LLINs), intermittent preventive treatment (IPT) of malaria, and preventive chemotherapy using albendazole or mebendazole against soil-transmitted helminthiases and praziquantel against schistosomiasis have been suggested (ter Kuile et al., 2003; Tolentino and Friedman, 2007; Rohner et al., 2010).

Previous studies suggest that >40% of the population in Côte d'Ivoire is anaemic and iron deficiency (ID) was identified as a moderate cause (Staubli-Asobayire et al., 2001; Wegmüller et al., 2006). However, a recent study conducted in south-central Côte d'Ivoire showed that, among iron fortification with electrolytic iron, IPT using sulfadoxine-pyrimethamine, and preventive chemotherapy using albendazole and praziquantel in school-aged children, preventive chemotherapy was the only intervention that had a small but significant effect on improving haemoglobin (Hb) concentration (Rohner et al., 2010).

The aetiology of anaemia has been investigated in various population groups across Africa using cross-sectional epidemiological designs, whereas only a handful of longitudinal studies assessed the dynamics of Hb throughout time in relation to specific factors (Cornet et al., 1998; Bloland et al., 1999; McElroy et al., 1999; Zimmermann et al., 2005). We designed a 14-month prospective longitudinal study, with repeated cross-sectional surveys conducted every 3-4 months, to assess the dynamics of Hb in relation to parasitic infections, micronutrient status, and increasing age in 3 cohorts in south-central Côte d'Ivoire.

5.3 Materials and methods

5.3.1 Ethical Considerations

The study was approved by the institutional research commissions of the Swiss Tropical and Public Health Institute and the Eidgenössische Technische Hochschule (ETH) Zurich. The ethics committees of Basel and Côte d'Ivoire approved the study. Investigators were covered by liability insurance. Village authorities, participants, and parents/guardians of minors were informed about the purpose, procedures, and potential risks and benefits of the study. Written informed consent (or fingerprints of illiterate people) was obtained from study participants and parents/guardians of infants and children aged 6-8 years. Participation was voluntary; hence one could withdraw from the study at any time without further obligations.

Clinical malaria cases (defined by a positive rapid diagnostic test (RDT) and a tympanic temperature $>38^{\circ}\text{C}$) were treated with artesunate-amodiaquine (Maphar, Sanofi-Aventis, Casablanca, Morocco). Soil-transmitted helminth and schistosome infections were treated with albendazole (GlaxoSmithKline) and praziquantel (Bayer), respectively. Severely anaemic participants (i.e., Hb <8 g/dl, according to national guidelines of Côte d'Ivoire) were referred to healthcare centres.

5.3.2 Study Design and Procedures

The setting, selection of study participants, and field and laboratory procedures have been described elsewhere (Righetti et al., 2012a; 2012b). In brief, a 14-month prospective longitudinal study was performed between April 2010 and June 2011, in 3 settings of the Taabo health demographic surveillance system (HDSS) in south-central Côte d'Ivoire. The 3 settings were Taabo Cité, a small district town where the only hospital is located; Ahondo, a village in close proximity to the Bandama River; and Katchénou, a small hamlet with no health facility. We set up the following 3 cohorts: infants aged 6-23 months; children of early school age (6-8 years); and young women aged 15-25 years. A total of 732 individuals were invited to participate.

At each cross-sectional survey, people were asked to provide stool and urine samples. Participants' height (to the nearest cm), weight (to the nearest 0.5 kg), and tympanic temperature (to the nearest 0.1°C) were measured. Finger-prick blood was collected and Hb concentration determined using a HemoCue 301 (HemoCue AB; Ängelholm, Sweden). Infection with *Plasmodium falciparum* was assessed using an RDT (ICT ML01 malaria Pf kit; ICT Diagnostics, Cape Town, South Africa). Additionally, thick and thin blood films were made to determine parasitaemia and species-specific *Plasmodium* infection. At the baseline

and end-of-study surveys, venous blood samples (5-10 mL) were drawn from each participant directly into heparin-coated tubes and put in a cool-box containing ice.

Duplicate Kato-Katz thick smears were prepared from each stool sample using 41.7 mg templates (Katz et al., 1972). The slides were allowed to clear for at least 30 min before microscopic examination by experienced laboratory technicians who recorded the number of eggs of soil-transmitted helminths (*Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*) and *Schistosoma mansoni*. Urine samples were subjected to a filtration method (WHO, 1991) counting all *S. haematobium* eggs in a filtrate of 10 ml. For quality-control, a senior laboratory technician reexamined 10% of Kato-Katz and urine filtration slides. Pregnancies and deliveries known by the participating women or by the community health workers were recorded during the study in order to adapt the cut-off used for anaemia in women.

5.3.3 Venous Blood Examination

Riboflavin was measured by an erythrocyte glutathione reductase activity coefficient (EGRAC) assay in whole blood (Dror et al., 1994). Ferritin, soluble transferrin receptor (sTfR), retinol binding protein (RBP), α 1-acid glycoprotein (AGP), and C-reactive protein (CRP) were measured with a sandwich enzyme-linked immunosorbant assay (ELISA) (Erhardt et al., 2004). Serum retinol (SR) was measured by high pressure liquid chromatography (Merck-Hitachi; Tokyo, Japan) (Tanumihardjo et al., 2004).

5.3.4 Statistical Analysis

Parasitological and haematological data were entered twice in Microsoft Access version 10.0 (2007 Microsoft Corporation) and cross-checked using EpiInfo version 3.4.1 (Centers for Disease Control and Prevention; Atlanta, GA, USA). Anaemia and storage iron depletion were defined according to World Health Organization (WHO) guidelines (WHO/UNICEF/UNU, 2001). Acute and chronic inflammation were defined as CRP >5 mg/L and AGP >1 g/L, respectively. Cellular ID was defined as sTfR >8.5 mg/L (Cook et al., 1993). Vitamin A deficiency was defined as RBP <0.825 μ mol/l (Gorstein et al., 2008).

Household socioeconomic status was calculated using an asset-based index (Filmer and Pritchett, 2001). Data on household assets, housing characteristics, and number of people per room were obtained from the readily available Taabo HDSS database. Using principal component analysis (PCA) to weigh the binary data of these variables, we subsequently divided the households into socioeconomic groups (wealth tertiles); namely, very poor, poor, and least poor.

Height-for-age and weight-for-height Z-scores were calculated with WHO AnthroPlus version 1.0.3 (WHO; Geneva, Switzerland). Stunting and underweight were defined as having a height-for-age, or weight-for-age, respectively, of more than two standard deviations (SD) below the median of the National Center for Health Statistics (NCHS)/WHO growth reference (WHO, 1995).

We employed logistic regressions to identify predictors of anaemia for those individuals who had complete parasitological, haematological, and micronutrients data at baseline or at the end-of-study survey. Crude and adjusted odds ratios (ORs) were calculated, including 95% confidence intervals (CIs) and *P* value using a Wald test. Setting was included as random effect in multivariate models, and covariates were removed until the best fitting model was identified, based on the results of a likelihood ratio test.

We used linear regression analyses with 2 clustering effects (ie, individual and setting level), to identify independent significant predictors of Hb, natural log sTfR, and natural log AGP. Individuals who had complete parasitological and haematological data for at least, 1 of the cross-sectional surveys were considered. For the models with sTfR and ferritin as outcomes, we included AGP only as marker of inflammation, to prevent colinearity with CRP and because AGP was better correlated to the outcome variables. For each outcome, we report the results of the best predictive model, based on Akaike's information criterion. McNemar test and Wilcoxon signed rank test were used to compare matched prevalence data and matched mean ranks, respectively.

5.4 Results

5.4.1 Attrition, participation, and socioeconomic parameters

Overall, 732 individuals were invited and, during the baseline cross-sectional survey, 407 (55.6%) were available and agreed to participate in the longitudinal monitoring (**Figure 5.1**). The participation rate decreased considerably from July 2010 onwards. An awareness campaign conducted in October 2010 stabilized the number of participants in the subsequent surveys despite a presidential post-electoral crisis that lasted until April 2011. An attrition analysis revealed that there was no difference in gender composition, median age, and socioeconomic status between participants with and without complete parasitological, haematological, and micronutrient data in April 2010 and in June 2011, regardless of the age group investigated.

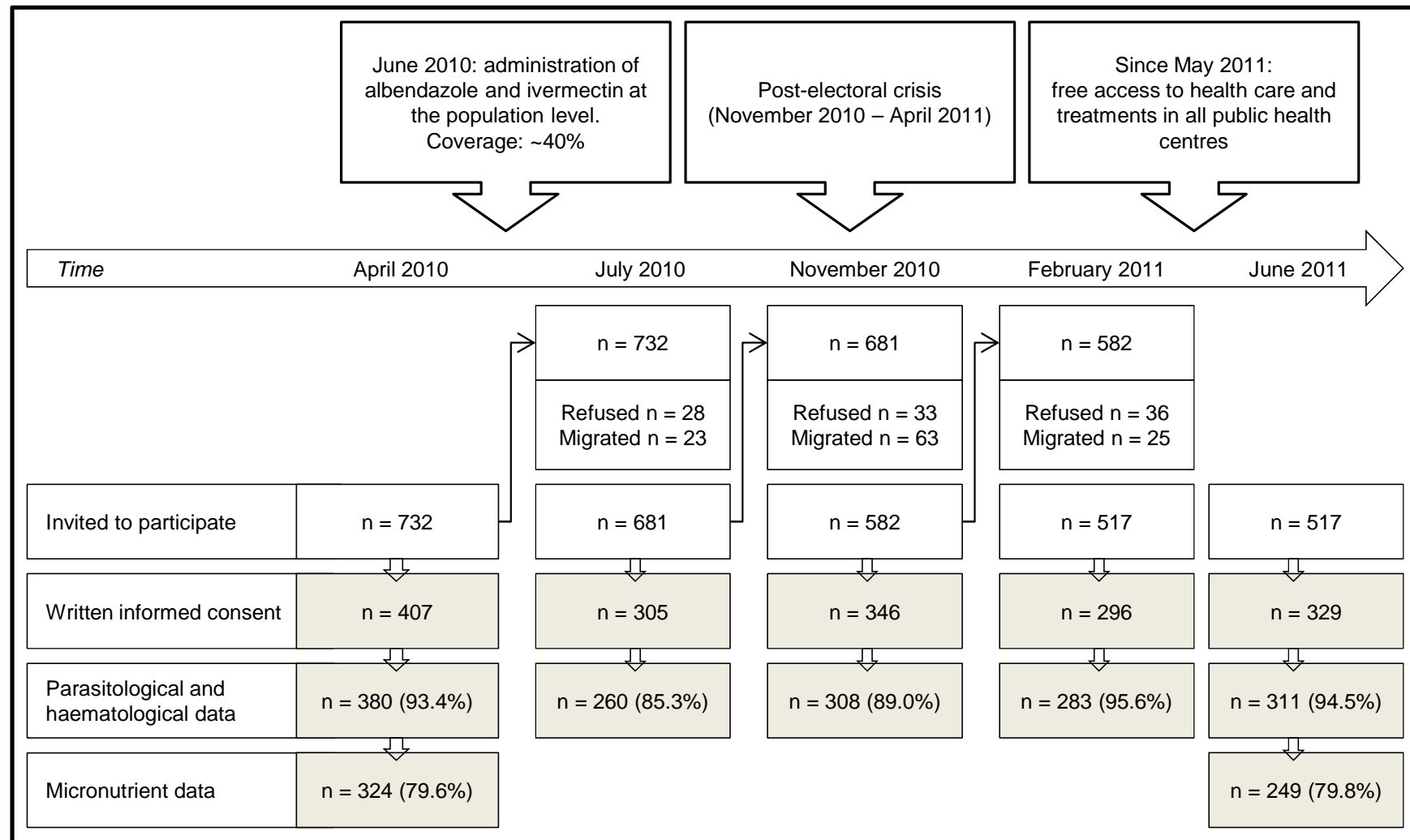


Figure 5.1: Participation, adherence, and major events during the 14-month longitudinal monitoring.

At the beginning of the study, in April 2010, 732 people were invited to participate, of whom 407 provided written informed consent for the longitudinal monitoring. All of the 732 initially invited people were again given the opportunity to participate in the second cross-sectional survey in July 2010. For the following rounds, only people who did not refuse to participate and did not move houses were asked for participation.

Moreover, median age and sex ratio of the 108 participants who had complete data for all five surveys did not significantly differ from the initial cohort of 732 individuals. The 3 settings were very different in terms of sociodemographic and economic parameters (**Table 5.1**).

Table 5.1: Socio-demographic parameters, household assets and other characteristics of infants, school-aged children, and women.

Characteristic	Taabo Cité		Ahondo		Katchénou		Difference	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	χ^2	<i>P</i> value
Sex (female)^a	38	44.7	40	47.6	60	51.3	0.87	0.647
Education (binary)								
Maternal education ^b	15	28.9	7	18.4	4	13.8	2.06	0.358
Attending school ^c	20	43.5	23	45.1	17	27.9	4.34	0.114
Ever attended school ^d	27	77.1	18	58.1	8	28.6	14.98	0.001
Mean number of people per room (categorical)								
1-1.9	33	21.7	43	37.1	55	49.1		
2-2.9	50	32.9	33	28.5	38	33.9		
≥ 3	69	45.4	40	34.5	19	17.0	30.50	<0.001
Household assets (binary)								
Ventilator	69	45.4	23	19.8	0	0.0	†	<0.001
Cell phone	136	89.5	88	75.9	82	73.2	13.19	0.001
Television	106	69.7	65	56.0	8	7.1	106.70	<0.001
Fridge	19	12.5	2	1.7	0	0.0	†	<0.001
Bicycle	98	64.5	93	80.2	99	88.4	21.79	<0.001
Car	3	2.0	0	0.0	0	0.0	†	0.116
Source of light (categorical)								
Candle	1	0.7	0	0.0	0	0.0		
Oil lamp	3	2.0	14	12.1	111	99.1		
Plugged lamp	46	96.1	102	87.9	0	0.0		
Other	2	1.3	0	0.0	1	0.9	†	<0.001
Source of water (categorical)								
Tap water	151	99.3	12	10.3	0	0.0		
Pump, well	1	0.7	59	50.9	112	100.0		
River	0	0.0	45	38.8	0	0.0	†	<0.001
Sanitation (categorical)								
Water closed	67	44.1	1	0.9	0	0.0		
Latrines	53	34.9	58	50.0	0	0.0		
No sanitation	32	21.1	57	49.1	112	100.0	†	<0.001

^a For infants and school-aged children only (*n* = 286)

^b For mothers of infants who answered the questionnaire (*n* = 105)

^c For school-aged children only (*n* = 158)

^d For young women only (*n* = 94)

† Fisher's exact test. Participants with complete parasitological data were included in the analyses, stratified by study setting (April 2010; *n* = 380)

5.4.2 Dynamics of parasitic infections, micronutrient status, and anaemia

Hb concentration was higher in June 2011 compared with April 2010 in the 3 cohorts (**Figure 5.2A**), and the change was significant for infants and school-aged children (**Table 5.2**). The intensity of *Plasmodium* parasitaemia slightly decreased during the long dry season (from October until March) in the 3 cohorts but increased with the start of the rainy season (**Figure 5.2C**). The geometric mean of soil-transmitted helminth and *Schistosoma* infections in school-aged children was significantly lower in June 2011 compared with the baseline cross-sectional survey done in April 2010 (Table 5.2). The geometric mean of *Schistosoma* infection at the end of the study was significantly lower among women compared with baseline prevalence data (Table 5.2). Iron status of infants significantly improved during the study, as reported by higher ferritin concentration and lower sTfR concentration in June 2011 compared with concentrations in April 2010 (Table 5.2).

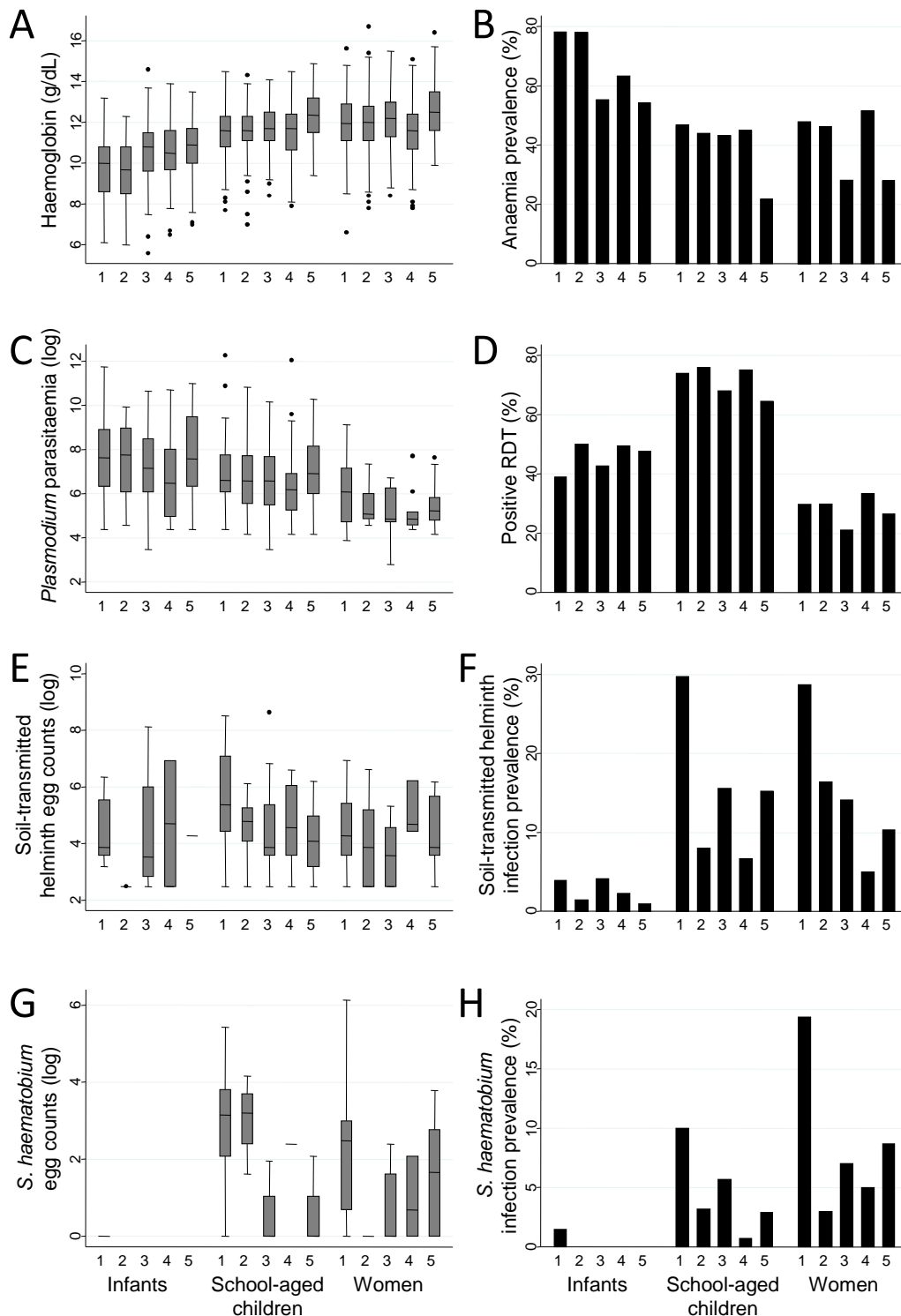


Figure 5.2: Anaemia and parasitic infection profiles over a 14-month longitudinal monitoring, stratified by age group.

A, dynamics of haemoglobin concentration in each age group; **B**, prevalence of anaemia; **C**, dynamics of *Plasmodium* parasitaemia in each age group; **D**, prevalence of *Plasmodium* infection; **E**, dynamics of soil-transmitted helminth infection intensity in each age group; **F**, prevalence of soil-transmitted helminth infection; **G**, dynamics of *S. haematobium* infection intensity in each age group, **H**, prevalence of *S. haematobium* infection. For each survey (1-5), all participants with complete parasitological and haematological data were included. April 2010 (1), $n = 380$; July 2010 (2), $n = 260$; November 2010 (3), $n = 308$; February 2011 (4), $n = 283$; June 2011 (5), $n = 311$. Box plot: the ends of the box represent the 75th and 25th percentiles; the middle line represents the median; the upper whisker represents the upper quartile + 1.5* (interquartile range); the lower whisker represents the lower quartile - 1.5* (interquartile range).

Table 5.2: Comparison of haematological, infection, inflammation and micronutrient parameters at baseline (April 2010), and at the end-of-study survey (June 2011), stratified by age group.

	Infants			Children			Women		
	April 2010	June 2011	<i>P</i> value	April 2010	June 2011	<i>P</i> value	April 2010	June 2011	<i>P</i> value
Haemoglobin (g/dl) ^{a†}	9.82 ± 0.17	10.76 ± 0.17	<0.001	11.53 ± 0.11	12.40 ± 0.11	<0.001	12.17 ± 0.26	12.60 ± 0.22	0.065
<i>P. falciparum</i> ^{b‡}	9.57 (3.42-24.27)	16.17 (5.97-41.34)	0.630	143.09 (78.93-258.74)	42.34 (21.71-81.72)	0.069	7.33 (1.99-22.21)	7.26 (2.48-18.61)	0.548
Soil-transmitted helminth ^{b‡}	0.19 (0.00-0.46)	0	0.083	3.21 (1.67-5.64)	0.87 (0.40-1.50)	0.002	2.66 (0.77-6.60)	0.47 (0.00-1.17)	0.081
<i>S. haematobium</i> ^{b‡}	0.00 (0.00-0.03)	0	0.317	0.46 (0.19-0.80)	0.04 (0.00-0.08)	0.006	0.90 (0.24-1.90)	0.02 (0.00-0.05)	0.003
sTfR (mg/l) ^{c‡}	11.50 (8.47-16.37)	8.95 (7.12-13.10)	0.001	7.88 (6.50-9.49)	7.50 (5.56-9.55)	0.100	7.22 (5.54-10.45)	6.54 (4.65-8.70)	0.233
Ferritin (µg/l) ^{c‡}	32.80 (15.00-69.20)	60.82 (29.92-96.84)	0.046	63.50 (45.80-103.80)	77.28 (47.20-105.36)	0.220	44.25 (27.35-60.60)	50.21 (24.70-69.61)	0.900
Serum retinol (µg/dl) ^{c‡}	19.06 (15.14-26.90)	22.38 (15.35-24.46)	0.602	21.20 (16.00-27.91)	23.18 (18.96-28.80)	0.003	34.53 (25.41-44.32)	37.31 (28.09-46.83)	0.635
EGRAC ^{c‡}	1.35 (1.18-1.50)	1.42 (1.32-1.62)	0.002	1.47 (1.29-1.71)	1.50 (1.39-1.67)	0.163	1.45 (1.28-1.65)	1.54 (1.41-1.66)	0.952
AGP (g/l) ^{c‡}	0.99 (0.84-1.33)	1.08 (0.90-1.33)	0.566	0.92 (0.77-1.06)	0.93 (0.66-1.05)	0.110	0.80 (0.70-0.94)	0.67 (0.52-0.89)	0.013
CRP (mg/l) ^{c‡}	2.18 (0.69-5.80)	1.20 (0.46-4.51)	0.650	1.57 (0.51-4.55)	1.72 (0.61-4.16)	0.514	1.04 (0.50-3.54)	1.06 (0.57-1.92)	0.456

^a Mean ± standard error^b Geometric mean (95% CI)^c Median (95% CI)[†] Bilateral paired t-test[‡] Wilcoxon signed rank testMatched parasitological and haematological data (*n* = 231), iron status and inflammation data (*n* = 189), serum retinol values (*n* = 163), and EGRAC (*n* = 211) are compared

Logistic regression revealed that variables significantly associated with anaemia in June 2011 differed from those identified in April 2010 in each age group (Tables 5.3, 5.4, and 5.5).

Table 5.3: Association of anaemia with socio-demographic, parasitic, and micronutrient status parameters in infants.

	Infants			
	April 2010 (n = 95)		June 2011 (n = 67)	
	OR	Adjusted OR (95% CI)	OR	Adjusted OR (95% CI)
Demographic variables				
Age class: middle ^a	1.3		1.3	
Age class: older ^a	1.3		0.6	
Sex (female)	0.9		0.7	
Socioeconomic status^b				
Poor	0.8		0.8	
Least poor	0.2		0.8	
Parasitic infection				
<i>P. falciparum</i>	3.8	3.8 (1.0-14.5)	2.8	
Soil-transmitted helminths	0.4		N/A	
<i>Schistosoma</i>	N/A		N/A	
Micronutrient deficiency				
Stunted (HAZ < 2)	1.1		2	
Underweight (WAZ < 2)	4.4		2.1	
Iron storage depletion	1.5		2.5	
Cellular iron deficiency	0.8		3.4	3.2 (1.0-9.7)
Vitamin A deficiency (RBP)	1.2		3.1	3.3 (1.0-10.9)
Riboflavin deficiency	0.7		0.5	
Other parameters				
Chronic inflammation	0.9		4.3	3.6 (1.2-10.9)
Acute inflammation	1.4		2.2	

^a Reference age class: youngest class. Subclasses: 6-month ranges.

^b Reference status: most poor.

Logistic regression was used to identify variables significantly associated with anaemia. Potential explanatory variables were: sex (binomial, where applicable), age (categorical), socioeconomic status (categorical), *Plasmodium falciparum* infection (binomial), soil-transmitted helminths infection (binomial), *Schistosoma* infection (binomial), iron storage depletion (binomial), cellular iron deficiency (binomial), vitamin A deficiency (RBP, binomial), riboflavin deficiency (binomial), chronic inflammation (binomial), acute inflammation (binomial). Multivariate regression model was computed with all potential covariates with setting included as random effect. In a first step, covariates were removed by stepwise backward procedure, keeping only explanatory variables with *P* values <0.20. In a second step, likelihood ratio test was applied to identify the best predictive model. We report adjusted odds ratios of those explanatory kept in the final model. Explanatory variables with crude and adjusted *P* values <0.05 are reported in bold. Abbreviations: CI, confidence interval; HAZ, height-for-age Z score; N/A, not applicable; RBP, retinol-binding protein; WAZ, weight-for-age Z score.

Table 5.4: Association of anaemia with socio-demographic, parasitic, and micronutrient status parameters in young school-aged children.

	Early school-aged children			
	April 2010 (n = 147)		June 2011 (n = 120)	
	OR	Adjusted OR (95% CI)	OR	Adjusted OR (95% CI)
Demographic factors				
Age class: middle ^a	1.3		1.4	
Age class: older ^a	0.8		1.1	
Sex (female)	1.1		0.7	
Attending school	0.5	0.5 (0.2-1.0)	0.5	
Socioeconomic status^b				
Poor	0.9		0.7	
Least poor	0.5		0.4	
Parasitic infection				
<i>P. falciparum</i>	2.8		1.8	
Soil-transmitted helminths	0.7		0.7	
<i>Schistosoma</i>	0.9		N/A	
Micronutrient deficiency				
Stunted (HAZ < 2)	2.5		5.9	5.8 (2.0-17.0)
Underweight (WAZ < 2)	2.1		4.4	
Iron storage depletion	2.4		N/A	
Cellular iron deficiency	2.9	2.3 (1.1-4.9)	1.7	
Vitamin A deficiency (RBP)	1.4		1.9	
Riboflavin deficiency	0.6		0.8	
Other factors				
Chronic inflammation	4.4	3.8 (1.8-8.3)	2	
Acute inflammation	3.2		1.6	3.0 (1.0-8.5)

^a Reference age class: youngest class. Subclasses: 1-year ranges.

^b Reference status: most poor.

Logistic regression was used to identify significant predictors of anaemia. Potential explanatory variables were: sex (binomial, where applicable), age (categorical), socioeconomic status (categorical), school attendance (for children, binomial), *Plasmodium* infection (binomial), soil-transmitted helminths infection (binomial), *Schistosoma* infection (binomial), iron storage depletion (binomial), cellular iron deficiency (binomial), vitamin A deficiency (RBP, binomial), riboflavin deficiency (binomial), chronic inflammation (binomial), acute inflammation (binomial). Multivariate regression model was computed with all potential covariates with setting included as random effect. In a first step, covariates were removed by stepwise backward procedure, keeping only explanatory variables with *P* values <0.20. In a second step, likelihood ratio test was applied to identify the best predictive model. We report odds ratios of all variables kept in the final model. Explanatory variables with crude and adjusted *P* values <0.05 are reported in bold. Abbreviations: HAZ, height-for-age Z score; N/A, not applicable; RBP, retinol-binding protein; WAZ, weight-for-age Z score.

Table 5.5: Association of anaemia with socio-demographic, parasitic, and micronutrient status parameters in young women.

	Young women			
	April 2010 (n = 82)		June 2011 (n = 62)	
	OR	Adjusted OR (95% CI)	OR	Adjusted OR (95% CI)
Demographic factors				
Age class: older ^a	0.4		0.9	
Occupation: student ^b	1.2	1.4 (0.4-4.9)	1.1	
Occupation: housekeeper ^b	0.3	0.2 (0.0-0.8)	1.4	
Attending school	N/A		N/A	
Socioeconomic status^c				
Poor	2.3		1.5	
Least poor	0.9		0.6	
Parasitic infection				
<i>P. falciparum</i>	1.2		0.9	
Soil-transmitted helminths	1.5		1.1	
<i>Schistosoma</i>	1.6		1.9	
Micronutrient deficiency				
Iron storage depletion	2.3		0.4	
Cellular iron deficiency	4.5	6.1 (1.9-19.6)	1.1	
Vitamin A deficiency (RBP)	N/A		N/A	
Riboflavin deficiency	0.4	0.3 (0.1-0.9)	1.9	
Other factors				
Chronic inflammation	1.1		1.7	
Acute inflammation	1.9		4.7	5.8 (1.2-28.0)

^a Reference age class: youngest class. Subclasses: 5-year ranges

^b Reference occupation: working out of the family house

^c Reference status: most poor

Logistic regression was used to identify significant predictors of anaemia. Potential explanatory variables were: age (categorical), socioeconomic status (categorical), occupation (categorical), *Plasmodium* infection (binomial), soil-transmitted helminths infection (binomial), *Schistosoma* infection (binomial), iron storage depletion (binomial), cellular iron deficiency (binomial), vitamin A deficiency (RBP, binomial), riboflavin deficiency (binomial), chronic inflammation (binomial), acute inflammation (binomial). Multivariate regression model was computed with all potential covariates with setting included as random effect. In a first step, covariates were removed by stepwise backward procedure, keeping only explanatory variables with *P* values < 0.20. In a second step, likelihood ratio test was applied to identify the best predictive model. We report odds ratios of all variables kept in the final model. Explanatory variables with crude and adjusted *P* values < 0.05 are reported in bold. Abbreviations: HAZ, height-for-age Z score; N/A, not applicable; RBP, retinol-binding protein; WAZ, weight-for-age Z score

At the end-of-study survey, the variables significantly associated with anaemia among infants were identical to those identified for young school-aged children at the beginning of the study. In June 2011, cellular ID and chronic inflammation were significantly associated with anaemia in infants while chronic malnutrition, defined as stunting, and acute inflammation were significant predictors of anaemia in school-aged children. At the end-of-study survey, acute inflammation was the only parameters significantly associated with lower Hb concentration.

Mixed-effect linear regression with 2 levels of clustering (individual and setting) revealed significant relationships between Hb concentration and age, *Plasmodium*, parasitaemia, and time in infants (**Table 5.6**). Plasmodium parasitaemia, *S. haematobium* egg counts, and chronic malnutrition (stunting) were significantly associated with Hb concentration in school-aged children. Among women, pregnancy status was significantly associated with lower Hb concentration. While mean Hb steadily increased over time in the infant cohort, this trend was less obvious in school-aged children and women (Table 5.6).

Table 5.6: Association between age, parasitic infections, micronutrient status and haemoglobin concentration.

Explanatory variables	Coefficient	95% CI	P value
Infants			
2 nd survey	-0.09	-0.45, 0.27	0.615
3 rd survey	0.61	0.24, 0.98	0.001
4 th survey	0.59	0.16, 1.03	0.008
5 th survey	0.68	0.17, 1.19	0.009
Age (longitudinal)	0.03	0.00, 0.06	0.026
<i>Plasmodium</i> parasitaemia	-0.15	-0.18, -0.12	<0.001
Stunted	-0.23	-0.48, 0.01	0.063
Intercept	9.76	9.26, 10.3	<0.001
Early school-aged children			
2 nd survey	-0.03	-0.26, 0.19	0.77
3 rd survey	0.16	-0.06, 0.38	0.15
4 th survey	-0.07	-0.29, 0.15	0.527
5 th survey	0.75	0.53, 0.97	<0.001
<i>Plasmodium</i> parasitaemia	-0.03	-0.06, -0.01	0.014
<i>S. haematobium</i> egg counts	-0.15	-0.28, -0.01	0.040
Stunted	-0.48	-0.80, -0.16	0.003
Intercept	11.81	11.58, 12	<0.001
Young women			
2 nd survey	0.05	-0.32, 0.42	0.798
3 rd survey	0.34	-0.03, 0.72	0.072
4 th survey	-0.28	-0.68, 0.12	0.166
5 th survey	0.59	0.22, 0.96	0.002
Pregnancy	-0.99	-1.40, -0.58	<0.001
<i>Plasmodium</i> parasitaemia	-0.04	-0.09, 0.01	0.112
Intercept	12.12	11.82, 12.4	<0.001

A mixed-effect linear regression was applied for each age group, with two levels of clustering (individual and setting). Potential explanatory variables were: survey (1-5), age (longitudinal), sex, *Plasmodium* parasitaemia (natural log-transformed), *S. haematobium* egg counts (natural log-transformed), soil-transmitted helminth egg counts (natural log transformed), stunting, and underweight for infants and school-aged children, and pregnancy status and weigh for women. The coefficients from the best predictive model, as defined by the Akaike's information criterion, are reported with their 95% confidence intervals (CI) and respective p-value.

The investigation of potential associations between parasitic infections and micronutrient biomarkers revealed that *Plasmodium* parasitaemia and AGP both significantly contributed to higher ferritin concentration in all age groups (Tables 5.7, 5.8, and 5.9). Furthermore, *S. haematobium* egg counts and EGRAC were significantly associated with lower ferritin concentration in the school-aged child cohort. The inflammatory marker AGP was significantly positively associated with sTfR concentration in the 3 age groups investigated (Tables 5.7, 5.8, and 5.9).

Table 5.7: Longitudinal analyses of variables associated with biomarkers of iron status and inflammation in infants.

Explanatory variables	Coefficient	95% CI	<i>P</i> value
Ferritin			
Age (in months)	0.03	0.01, 0.04	<0.001
Sex (female)	0.26	0.02, 0.49	0.036
<i>Plasmodium</i> parasitaemia	0.09	0.06, 0.12	<0.001
Stunted	-0.13	-0.35, 0.09	0.260
AGP	0.92	0.54, 1.30	<0.001
Intercept	2.80	2.48, 3.12	<0.001
sTfR			
5 th survey	-0.08	-0.11, -0.04	<0.001
Stunted	0.19	0.01, 0.37	0.037
AGP (log)	0.33	0.05, 0.61	0.020
Intercept	2.48	2.32, 2.64	<0.001
AGP			
5 th survey	-0.08	-0.16, 0.00	0.061
<i>Plasmodium</i> parasitaemia	0.04	0.03, 0.05	<0.001
EGRAC	0.17	-0.07, 0.40	0.175
Intercept	-0.04	-0.13, 0.06	0.455

A mixed-effect linear regression was applied, with two levels of clustering (individual and setting). Potential covariates were: age (numerical), sex (binary), survey (1 or 5), *Plasmodium* parasitaemia (natural log-transformed), *S. haematobium* egg counts (natural log-transformed), soil-transmitted helminth egg counts (natural log transformed), EGRAC (natural log-transformed), serum retinol (natural log-transformed) and, for the models with ferritin or sTfR as outcome (natural log-transformed), AGP (natural log-transformed). The coefficients from the best predictive model, as defined by the Akaike's information criterion, are reported with their 95% confidence intervals (CI) and respective *P* value.

Table 5.8: Longitudinal analyses of variables associated with biomarkers of iron status and inflammation in young school-aged children.

Explanatory variables	Coefficient	95% CI	<i>P</i> Value
Ferritin			
5 th survey	0.18	0.10, 0.27	<0.001
<i>Plasmodium</i> parasitaemia	0.03	0.01, 0.04	0.001
<i>S. haematobium</i> egg counts	-0.13	-0.20, -0.06	<0.001
AGP	0.88	0.69, 1.08	<0.001
EGRAC	-0.68	-1.03, -0.33	<0.001
Intercept	4.41	4.24, 4.58	<0.001
sTfR			
5 th survey	-0.02	-0.04, -0.01	0.007
Soil-transmitted helminth egg counts	-0.01	-0.03, 0.00	0.158
AGP	0.55	0.41, 0.68	<0.001
Serum retinol	0.13	0.01, 0.25	0.034
Intercept	1.79	1.43, 2.15	<0.001
AGP			
<i>Plasmodium</i> parasitaemia	0.02	0.01, 0.03	<0.001
Soil-transmitted helminth egg counts	-0.01	-0.03, 0.00	0.061
EGRAC	-0.17	-0.36, 0.02	0.085
Intercept	-0.14	-0.24, -0.05	0.003

A mixed-effect linear regression was applied, with two levels of clustering (individual and setting). Potential covariates were: age (numerical), sex (binary), survey (1 or 5), *Plasmodium* parasitaemia (natural log-transformed), *S. haematobium* egg counts (natural log-transformed), soil-transmitted helminth egg counts (natural log-transformed), EGRAC (natural log-transformed), serum retinol (natural log-transformed) and, for the models with ferritin or sTfR as outcome (natural log-transformed), AGP (natural log-transformed). The coefficients from the best predictive model, as defined by the Akaike's information criterion, are reported with their 95% confidence intervals (CI) and respective *P* value.

Table 5.9: Longitudinal analyses of variables associated with biomarkers of iron status and inflammation in women.

Explanatory variables	Coefficient	95% CI	<i>P</i> Value
Ferritin (log)			
<i>Plasmodium</i> parasitaemia	0.04	0.00, 0.07	0.045
Soil-transmitted helminth egg counts)	0.03	-0.01, 0.08	0.158
<i>S. haematobium</i> egg counts	-0.09	-0.18, 0.00	0.056
AGP (log)	0.88	0.59, 1.18	<0.001
Intercept	3.96	3.77, 4.16	<0.001
sTfR			
<i>Plasmodium</i> parasitaemia	-0.02	-0.05, 0.00	0.059
AGP	0.22	0.01, 0.43	0.042
Serum retinol	-0.18	-0.35, -0.01	0.037
EGRAC	0.28	-0.11, 0.68	0.163
Intercept	2.63	2.02, 3.24	<0.001
AGP (log)			
Pregnancy	-0.44	-0.64, -0.24	<0.001
<i>Plasmodium</i> parasitaemia	0.02	0.00, 0.04	0.024
Intercept	-0.34	-0.40, -0.28	<0.001

A mixed-effect linear regression was applied, with two levels of clustering (individual and setting). Potential covariates were: age (numerical), sex (binary), survey (1 or 5), *Plasmodium* parasitaemia (natural log-transformed), *S. haematobium* egg counts (natural log-transformed), soil-transmitted helminth egg counts (natural log transformed), EGRAC (natural log-transformed), serum retinol (natural log-transformed) and, for the models with ferritin or sTfR as outcome, AGP (natural log-transformed). The coefficients from the best predictive model, as defined by the Akaike's information criterion, are reported with their 95% confidence intervals (CI) and respective *P* value.

Natural log sTfR was significantly correlated with natural log AGP (Spearman's ρ : 0.43; P <0.001) and natural log CRP (Spearman's ρ : 0.22; P <0.001) (**Figures 5.3A and 5.3B**). Natural log sTfR was correlated to natural log AGP (Spearman ρ : 0.22; P <0.001) and CRP concentration (Spearman ρ : 0.40; P <0.001) in participants free of *Plasmodium* infection as well (data not shown). At baseline, participants with chronic or acute inflammation had significantly higher sTfR concentration than their counterparts without inflammation (**Figures 5.3C and 5.3D**). Natural log sTfR was significantly lower in June 2011 compared with that in April 2010 in participants with inflammation at baseline and no inflammation at the end-of-study survey (**Figures 5.3E and 5.3F**). Our data indicate that serum retinol concentration was inversely correlated with sTfR in school-aged children and women (Tables 5.7, 5.8, and 5.9). *Plasmodium* parasitaemia was associated with higher AGP values in the 3 cohorts.

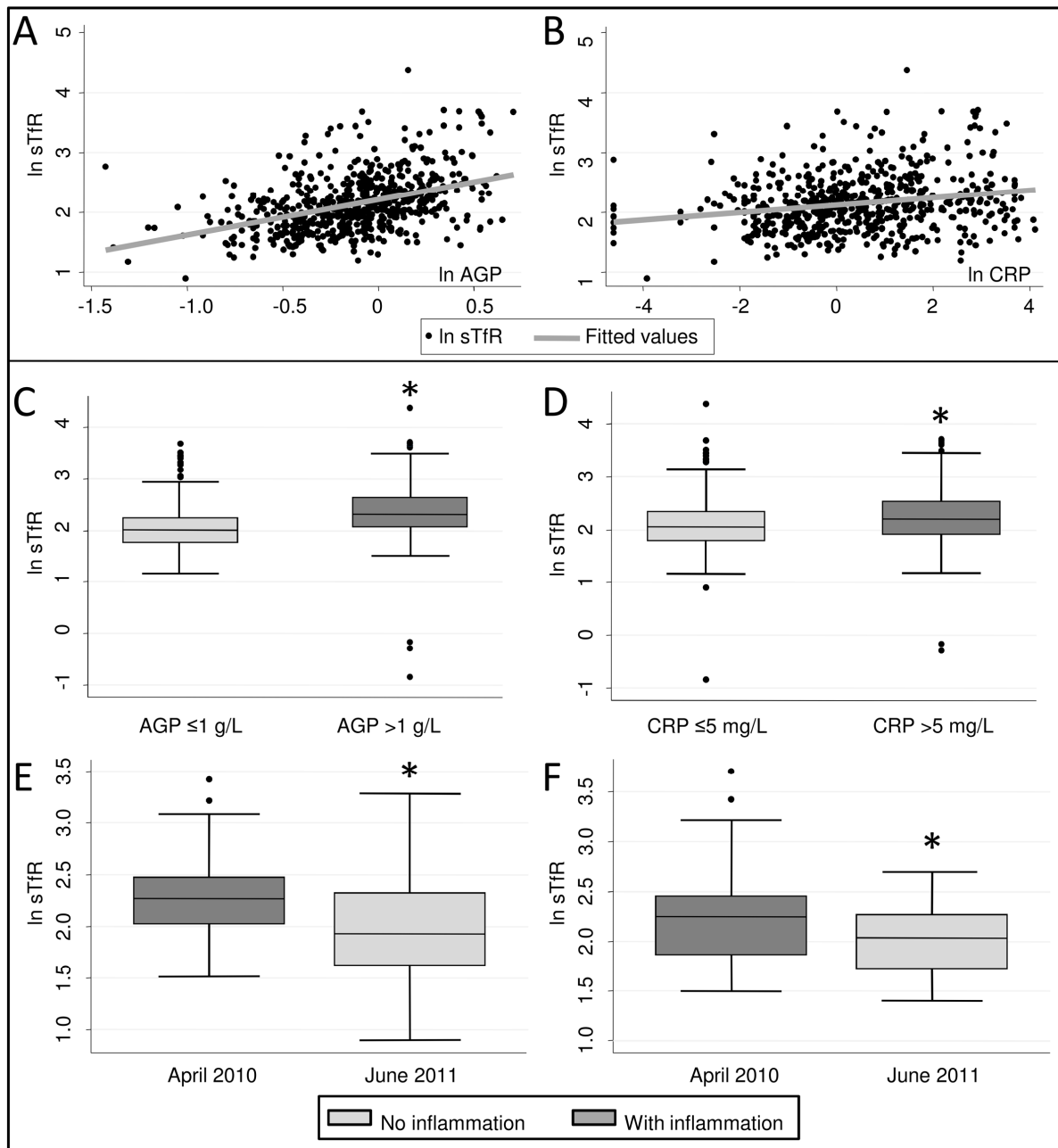


Figure 5.3: Association between inflammatory biomarkers and sTfR.

A, scatter and fitted plot of natural log sTfR values and log AGP ($n = 610$; 3 outliers with log sTfR < 0 are not shown); **B**, scatter and fitted plot of natural log sTfR values and log CRP ($n = 610$; 3 outliers with log sTfR < 0 are not shown); **C**, comparison of natural log sTfR between individuals with and without chronic inflammation ($n = 344$; April 2010); **D**, comparison of natural log sTfR between individuals with and without acute inflammation ($n = 344$; April 2010); **E**, comparison of natural log sTfR within individuals with and without chronic inflammation ($n = 38$); **F**, comparison of natural log sTfR within individuals with and without acute inflammation ($n = 33$). Asterisks indicate Wilcoxon rank-sum or signed-rank (for paired data) P values < 0.05 .

5.5 Discussion

To our knowledge, this is the first prospective longitudinal survey to investigate the dynamics of anaemia and putative associated factors over a 14-month period, in 3 cohorts in tropical West Africa. Our data indicate that infection with *Plasmodium* and *Schistosoma*, cellular ID, chronic malnutrition and inflammation are significantly associated with lower Hb concentration in the current, primarily rural, setting of south-central Côte d'Ivoire. The observation that sTfR concentration depend both on AGP and CRP concentration in the 3 cohorts investigated challenges the robustness of this marker to assess the prevalence of ID in areas where inflammatory diseases are common.

Our longitudinal survey revealed higher Hb concentration among infants and school-aged children at the end-of-study survey in June 2011 compared with the concentration at the baseline cross-sectional survey April 2010. This difference can be explained, at least partially, by lower *S. haematobium* and *P. falciparum* infection intensities in school-aged children and by older age and improved iron status in infants at the end-of-study survey. However, some survey time points were significantly associated with haemoglobin concentration (see Table 4), indicating the influence of other factors not investigated here or that the study itself contributed to a decline in anaemia prevalence. We observed a slight decrease in Hb concentration in February 2011, which might be a consequence of one or several factors. First, this survey was done during the primary dry season, a period characterised by restricted food supply and poor diet diversification. Second, the socio-political unrest and armed conflict that Côte d'Ivoire went through in connection with the presidential post-election turmoil between November 2010 and April 2011 had an impact on population diet as well (Bonfoh et al., 2011). Indeed, access to a diversified diet not only became more difficult due to the cessation of food exchanges between rural and urban areas, but many residents from the economic capital Abidjan sought shelter in their home villages at the height of the conflict, possibly decreasing the relative amount of food available per person. Moreover, access to health care was compromised during this period. Third, among women, infections with *Plasmodium* were more prevalent in the February 2011 cross-sectional survey than in the preceding survey in November 2010 and the end-of-study survey in June 2011.

The identification of variables significantly associated with anaemia and parameters significantly associated with lower Hb concentration in each age group confirms the multifactorial aetiology of anaemia in the current setting of rural West Africa. Importantly, our data indicate that the variables associated with anaemia in infants and school-aged children

shifted during the study. Indeed, we found the same risk factors for anaemia as infants grew older (i.e., 20-38 months) as observed in young school-aged children (6-8 years).

Our data indicate that, for the infant cohort at the baseline cross-sectional survey, the result of an RDT for malaria is the single most accurate predictor for the odds of anaemia. The relationship between *Plasmodium* infection and parasitaemia on the one hand, and Hb and iron status parameters (i.e., ferritin and sTfR), on the other hand, was marked in each of the 3 cohorts and confirms the important burden of malarial anaemia in these age groups, in areas where malaria is highly endemic (Snow et al., 1999; Desai et al., 2007; Magalhães and Clements, 2011). However, the partial immunity that develops against *Plasmodium* in people living in malaria-endemic areas is reflected in our data by the significant relationship between *Plasmodium* infection and anaemia in infants only and the weak association between *Plasmodium* parasitaemia and Hb concentration in school-aged children and women (Langhorne et al., 2008). Of note, at the time of our study, coverage rates with LLINs in Côte d'Ivoire were very low (i.e., <10% of children aged <5 years slept under LLINs) (Noor et al., 2009).

Our data confirm that ID contributes to the burden of anaemia in sub-Saharan Africa, as well. Indeed, cellular ID was significantly associated with anaemia in infants, school-aged children, and women, although infants showed markedly higher prevalence of ID in our study. Other studies have reported a low prevalence of ID in school-aged children in this region of Côte d'Ivoire (Staubli-Asobayire et al., 2001; Wegmüller et al., 2006; Rohner et al., 2010), which could be due to a diet largely based on cassava and yams and thus with a low phytate content. Furthermore, among infants, iron status was the only parameter which significantly improved during the study, coupled to increased Hb concentration. Considering that *Plasmodium* infection decreases iron absorption, and in view of adverse events that may result from mass iron supplementation in malaria-endemic settings, high LLIN coverage, improved access to prompt diagnosis, and quality malaria treatment should precede potential iron fortification campaigns in Côte d'Ivoire (Menendez et al., 1997; Sazawal et al., 2006; Cercamondi et al., 2010).

Our prospective longitudinal monitoring highlights the fact that, in addition to *Plasmodium* infection and ID, chronic malnutrition and inflammation are significantly associated with anaemia in infants and young school-aged children in this area of West Africa (Magalhães and Clements, 2011). The inflammation induced by *Plasmodium* parasites was obvious in all cohorts studied, as reflected by the association between *Plasmodium* parasitaemia and AGP concentration, illustrating one of the mechanisms implicated in malarial anaemia (Chang and Stevenson, 2004). The overall low prevalence and intensities of helminth infections in the

study area might explain why we did not find any significant association between soil-transmitted helminth infection and Hb concentration in any age group. Indeed, although soil-transmitted helminth infection prevalence significantly decreased in school-aged children, most likely explained by the administration of anthelmintic drugs at the individual and the population levels, this parameter was not significantly associated with Hb concentration in this age group. However, our data confirm the negative association between *S. haematobium* egg counts, on the one hand, and iron stores and Hb on the other hand, in school-aged children and women (Prual et al., 1992; Olsen et al., 1998)

In addition, we found that *Plasmodium* parasitaemia and inflammation were significantly correlated with ferritin and sTfR concentration, respectively. Ferritin, which is a positive acute-phase protein, has been associated with *Plasmodium* infection in previous studies, confirming the current observation (Stoltzfus et al., 1997). However, the regulation of sTfR is less well understood, particularly in the context of inflammation. During the last decade, sTfR became a popular proxy for assessing iron status at the population level, particularly in regions where inflammatory diseases are prevalent (Staubli-Asobayire et al., 2001; Koulaouzidis et al., 2009). However, 2 studies carried out in Kenya reported higher sTfR levels in *Plasmodium*-infected children (Verhoef et al., 2001; Grant et al., 2012). Interestingly, in a study conducted in Benin, *Plasmodium*-infected women showed higher concentrations of sTfR after having received a malarial treatment, most likely reflecting the suppression of erythropoiesis, which occurs during chronic *Plasmodium* infection (Cercamondi et al., 2010). Our results indicate that, while the inflammatory marker AGP showed a significant positive correlation with sTfR concentration in all cohorts, *Plasmodium* parasitaemia was negatively correlated with sTfR concentration in women, most likely due to a decreased erythropoiesis rate in this age group. Moreover, our results show that sTfR concentration significantly differs between and among people with and without inflammation. These findings suggest that sTfR is also influenced by inflammation. Hence, it is likely that, in our setting, high sTfR may, in part, reflect functional ID that results from the prevention of intestinal iron absorption and iron recycling from macrophages by hepcidin (Ganz and Nemeth, 2012), rather than inadequate dietary iron supply. Future studies should include hepcidin measures, weighed food record, and quantitative food recalls in order to better understand the regulation of sTfR expression and the source of cellular ID.

During the April 2010 baseline cross-sectional survey done, we found that women with riboflavin deficiency had lower odds of anaemia (Righetti et al., 2012). However, the negative correlation between ferritin concentration and EGRAC observed in school-aged children emphasizes the association of iron handling and riboflavin status. Our findings are in line with previous results from a study done in the same area that reported a detrimental effect of

riboflavin deficiency on iron status among school-aged children (Powers, 2003; Rohner et al., 2007). Although previous laboratory investigations and clinical trials showed a positive effect of vitamin A on Hb concentration, iron mobilization, and iron absorption, the effect on ferritin and sTfR concentration was less well characterised (Mejia and Chew, 1988; Garcia-Casal et al., 1998; Walczyk et al., 2003; Jiang et al., 2012). The observation that vitamin A and sTfR are inversely correlated in children and women reflects this uncertain interaction and emphasizes the need of further research in order to gain a better understanding of nutrient-nutrient interactions and interplay between micronutrients and parasitic infections (Taylor and Higgs, 2000).

The current prospective longitudinal study sheds light on the complex aetiology of anaemia in rural, tropical West Africa. Most importantly, our data show that parasitic infections (e.g., malaria and schistosomiasis), inflammatory diseases, cellular ID, and chronic malnutrition are associated with lower Hb concentration. These considerations emphasize the urgent need to implement efficient prevention and control programs that target these parasitic diseases. Concerted efforts must include distribution of LLINs, anthelmintic drug administration and collaborative efforts to facilitate a more equitable access to a diversified diet. This diet should include an adequate intake of bioavailable iron, especially for infants, coupled to health and nutritional education, which is mandatory to achieve a sustainable impact on anaemia in sub-Saharan Africa.

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6. Effects of inflammation and *Plasmodium falciparum* infection on soluble transferrin receptor and plasma ferritin concentration in different age groups: a prospective longitudinal study in Côte d'Ivoire

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6.1 Abstract

Background: Iron deficiency (ID) is a major cause of anaemia, along with other nutritional, parasitic, and genetic factors. Accurate biomarkers are needed to estimate the relative contribution of ID to anaemia. Soluble transferrin receptor (sTfR) is thought to be unaffected by inflammation.

Objective: To determine the difference in sTfR and plasma ferritin (PF) concentrations among infants (6-23 mo), school-aged children (6-8 y), and women (15-25 y) with and without inflammation with and without *Plasmodium falciparum* infection, and to assess the effect of adjusting sTfR and PF for inflammation of for *P. falciparum* infection on the estimated prevalence of ID.

Design: The data stem from a 14-mo prospective longitudinal survey on anaemia, conducted in the Taabo area, south-central Côte d'Ivoire.

Results: At baseline, sTfR concentration was significantly higher in infants and school-aged children with either inflammation or *Plasmodium* infection than in control individuals without inflammation or without *P. falciparum* infection. Individuals with inflammation had significantly higher PF concentration than subjects without inflammation. Adjusting sTfR concentration for inflammation or *P. falciparum* infection in infants and school-aged children resulted in significantly lower ID prevalence. Adjusting PF for inflammation and *P. falciparum* infection resulted in higher ID prevalence in infants and women.

Conclusion: In Ivorian infants and school-aged children, ID prevalence was considerably lower after correcting sTfR for inflammation. However, as the prevalence estimates for ID differed widely if based on sTfR or PF, caution is still needed when estimating ID prevalence in areas with high prevalence of inflammation or malaria. This study is registered at controlled-trials.com as ISRCTN02181959.

6.2 Introduction

Anaemia is a serious public health problem worldwide, as one in four people live with this condition (McLean et al., 2009). The burden of anaemia is highest in low-income countries, where up to 60% of children aged <5 y and 45% of non-pregnant women are affected. In sub-Saharan Africa, anaemia is multifactorial, including nutritional, parasitic, and genetic factors (van den Broek and Letsky, 2000; Crawley, 2004; Atkinson et al., 2006; Tolentino and Friedman, 2007; Calis et al., 2008; Cardoso et al., 2012; Righetti et al., 2012). Iron deficiency (ID) was long accepted as the major etiological agent of anaemia, partly because haemoglobin (Hb) has been widely used as a proxy to estimate the prevalence of ID. However, first stages of ID do not affect Hb synthesis and there are forms of anaemia which are not caused by ID (WHO/UNICEF/UNU, 2001). In order to estimate the prevalence of ID and to quantify the differential contribution of ID to anaemia, there is a need for reliable biomarkers of iron status with a high sensitivity and specificity (Lynch, 2011).

Ferritin, a protein ubiquitously expressed in the human body that is responsible for iron sequestration, is the classical biomarker used to estimate body storage iron (Knovich et al., 2009). In the absence of inflammation, the concentration of plasma ferritin (PF) reflects body iron reserves (Cook, 2005). However, PF is also a positive acute-phase protein, which renders its utilization as a marker of body iron stores challenging in individuals affected by inflammation (Torti and Torti, 2002). The soluble form of the transferrin receptor (sTfR) - identified for the first time in serum in 1986 (Kohgo et al., 1986) - has become a widely used marker of ID in epidemiological studies (Staubli-Asobayire et al., 2001; Cercamondi et al., 2010; Shinoda et al., 2012). Of note, sTfR is expressed by iron-requiring cells (mainly erythroid precursors) and reflects cellular iron status and erythropoietic activity (Speeckaert et al., 2010). Previous studies have suggested that haemolytic and megaloblastic anaemias, but not inflammation, might significantly alter sTfR concentration (Verhoef et al., 2001; Zimmermann, 2008; Ayoya et al., 2010). However, two recent studies reported higher sTfR concentration in individuals affected by subclinical inflammation (Grant et al., 2011; Kasvosve et al., 2006).

Within the frame of a 14-mo prospective longitudinal study to assess the dynamics of anaemia in three cohorts (infants, school-aged children, and young women) in south-central Côte d'Ivoire, we found that, among other variables, cellular ID (as estimated by sTfR >8.5 mg/L (Staubli-Asobayire et al., 2001; Cook et al., 1993)) was associated with increased odds of anaemia (Righetti et al., 2012b; Righetti et al., 2013). However, inflammation was also significantly associated with anaemia in the three age groups studied and iron status biomarkers and inflammatory biomarkers correlated significantly.

The objective of this study was to expand on those initial observations, to determine the relationship between inflammation, *P. falciparum* infection, sTfR, and PF concentrations in different age groups at the baseline and at the end-of-study cross-sectional surveys, and to assess the effect of adjusting sTfR and PF for inflammation or *P. falciparum* infection on the estimated prevalence of ID.

6.3 Subjects and methods

6.3.1 Study area, design, and population surveyed

The data for the current study stem from a 14-mo prospective longitudinal survey on the aetiology of anaemia, conducted in the Taabo health demographic surveillance system (HDSS) in south-central Côte d'Ivoire (Becker et al., 2011; Fürst et al., 2012; Righetti et al., 2012a Righetti et al., 2012b; Righetti et al., 2013). The baseline cross-sectional survey was conducted in April 2010, with repeated cross-sectional follow-up surveys every 3-4 mo. The end-of-study survey was carried out in June 2011 (**Figure 6.1**).

At the beginning of the study, 732 participants were selected from the readily available database of the Taabo HDSS. We focused on three population groups: 1) infants (aged 6-23 mo; $n = 237$); 2) young school-aged children (6-8 y; $n = 215$); and 3) young women (aged 15-25 y; $n = 280$) from Taabo Cité (only small town of the area), Ahondo (village), and Katchénou (hamlet). Selected subjects were invited to participate in each of the cross-sectional surveys, even if they missed the baseline or any follow-up survey.

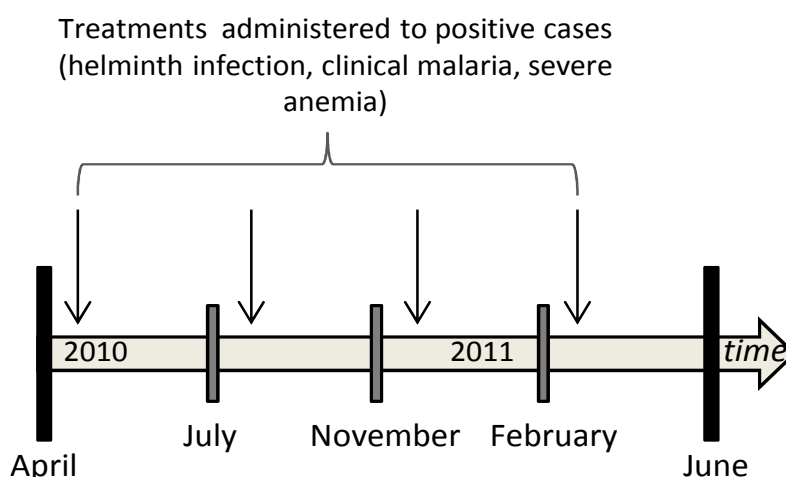


Figure 6.1: Design of the prospective longitudinal monitoring conducted among infants, school-aged children and young women in the Taabo health demographic surveillance system, south-central Côte d'Ivoire, between April 2010 and June 2011.

The study protocol was approved by the institutional research commissions of the Swiss Tropical and Public Health Institute (Basel, Switzerland; reference no. FK 96) and the Eidgenössische Technische Hochschule (ETH) Zurich (Zurich, Switzerland; reference no. EK 2009-N-19), and the ethics committees of Basel (EKBB; reference no. 252/09) and Côte d'Ivoire (reference no. 1086 MSHP/CNER). Investigators, the study physician and laboratory technicians were covered by liability insurance (GNA Assurance; Abidjan, Côte d'Ivoire). The purpose, procedures, potential risks, and benefits of the study were explained to village authorities and participating households. Written informed consent (or fingerprints of illiterate people) was obtained from study participants and parents/guardians of infants and school-aged children. Participation was voluntary, and hence one could withdraw from the study at any time with no further obligations.

6.3.2 Procedures

At each survey, participants were registered, height and weight measured, a finger-prick blood sample taken, and Hb concentration determined using a HemoCue 301 (HemoCue AB; Ängelholm, Sweden). Infection with *P. falciparum* was diagnosed using a rapid diagnostic test (RDT; ICT ML01 malaria Pf kit; ICT Diagnostics, Cape Town, South Africa). *Plasmodium* species-specific parasitaemia was determined based on microscopic examination of Giemsa-stained thick and thin blood films. Soil-transmitted helminths and *Schistosoma mansoni* infections were diagnosed with the Kato-Katz technique, whereas *Schistosoma haematobium* infection was determined by the urine filtration method (Katz et al., 1972; WHO, 1991). Venous blood samples (5-10 mL) were drawn from each participant at baseline and at the end-of-study survey directly into heparin-coated tubes and stored in a cool-box containing ice, pending transfer to the laboratory in Taabo Cité. Plasma was separated, aliquoted, and samples stored at -20°C. The plasma samples were shipped on dry ice to ETH Zurich, Switzerland and subsequently transferred to Freiburg, Germany for laboratory analyses of sTfR, PF, α 1-acid glycoprotein (AGP), and C-reactive protein (CRP) by using a sandwich enzyme-linked immunosorbant assay (ELISA) (Erhardt et al., 2004). Each value represents the mean of an independent duplicate measurement. Inter- and intra-assay coefficients of variation were <9%.

At each cross-sectional survey, suspected clinical malaria cases (defined by a positive RDT plus tympanic temperature >38°C) were provided with artesunate-amodiaquine (infants: 25 mg + 67.5 mg/day for 3 days; children: 100 mg + 270 mg/day for 3 days; women: 2 x 100 mg + 270 mg/day for 3 days; Maphar, Sanofi-Aventis, Casablanca, Morocco). Non-pregnant subjects with soil-transmitted helminth infection (i.e., *Ascaris lumbricoides*, hookworm, *Trichuris trichiura*), as determined by at least one helminth egg on Kato-Katz thick smears,

were given albendazole (400 mg for children and women and 200 mg for infants; GlaxoSmithKline). School-aged children (aged 6-8 y) and non-pregnant women with *S. mansoni* or *S. haematobium*, as determined by urine filtration or microscopic examination of two Kato-Katz thick smears, were treated with praziquantel (40 mg/kg; Bayer), respectively. Severely anaemic participants (i.e., Hb <80 g/L, according to national guidelines of Côte d'Ivoire) with a positive RDT for malaria were treated with artesunate-amodiaquine or were referred to the nearest health centre if their RDT was negative. These people were still eligible for enrolment.

6.3.3 Statistical analysis

Anaemia was defined according to guidelines put forth by the World Health Organization (WHO) (WHO/UNICEF/UNU, 2011). As many women became pregnant during the 14-mo of our longitudinal monitoring, we did not exclude pregnant women but adapted the cut-off used for anaemia, as proposed by the WHO. The thresholds used for defining abnormal values of the iron status and inflammation biomarkers were as follows: sTfR >8.3 mg/L (cut-off usually used for measures done with the ELISA from Ramco Laboratories and another in-house ELISA) (Grant et al., 2011; Erhardt et al., 2004) or >8.5 mg/L (cut-off used for minimizing the potential overestimation of ID prevalence and for comparing our results with previous findings from Côte d'Ivoire) (Staubli-Asobayire et al., 2001; Cook et al., 1993; Rohner et al., 2010); PF <12 µg/L (infants) or <15 µg/L (children and women) (WHO/UNICEF/UNU, 2001); CRP >5 mg/L; and AGP >1 g/L. Height-for-age (HAZ) and weight-for-age (WAZ) Z-scores were calculated with the WHO AnthroPlus version 1.0.3 (WHO; Geneva, Switzerland). Body mass index (BMI) was calculated for women as (weight (kg)) / (size (m²)). We defined four inflammatory states: 1) no inflammation (CRP ≤5 mg/L and AGP ≤1 g/L); 2) incubation (CRP >5 mg/L and AGP ≤1 g/L); 3) early convalescence (CRP >5 mg/L and AGP >1 g/L); and 4) late convalescence (CRP ≤5 mg/L and AGP >1 g/L) (Grant et al., 2011; Thurnham et al., 2010).

We used STATA version 10 (StataCorp.; College Station, TX, USA) for statistical analysis. Participants who had values for all of the aforementioned parameters at baseline ($n = 388$) or at the end-of-study survey ($n = 265$), with a tympanic temperature ≤38°C and sTfR concentration ≥1mg/l were considered for statistical analyses (**Figure 6.2**). Spearman's rank correlation coefficients were calculated with natural log-transformed values of AGP, CRP, sTfR, PF, and *Plasmodium* parasitaemia because the distribution of these variables were skewed, as defined by p-values of skewness and Kurtosis tests for normality <0.05. Means ± standard errors (SE), medians ± interquartile ranges, and geometric means (GM) ± standard deviations (SD) are reported. The sTfR/ferritin index was calculated by dividing sTfR (mg/L)

by log PF ($\mu\text{g/L}$) and the cut-off of ID was set to 5.3 (Phiri et al., 2009). We calculated the ratios of the GM values of the iron indicator for the group with inflammation, or with *P. falciparum* infection, to the reference group without inflammation, or free of *P. falciparum* infection, respectively (Thurnham et al., 2010). The correction factor (CF) was calculated as 1/ratio. We adjusted iron status indicator concentrations by multiplying the measured individual values by their group-specific CF (Grant et al., 2011). We used Wilcoxon's rank-sum and Kruskal Wallis' tests to assess for equality of sTfR concentration between two or several groups, respectively. Mc Nemar's chi square (χ^2) statistics were employed to compare the estimated prevalence based on non-adjusted values with prevalence calculated with adjusted values. Statistical significance was set to $P < 0.05$.

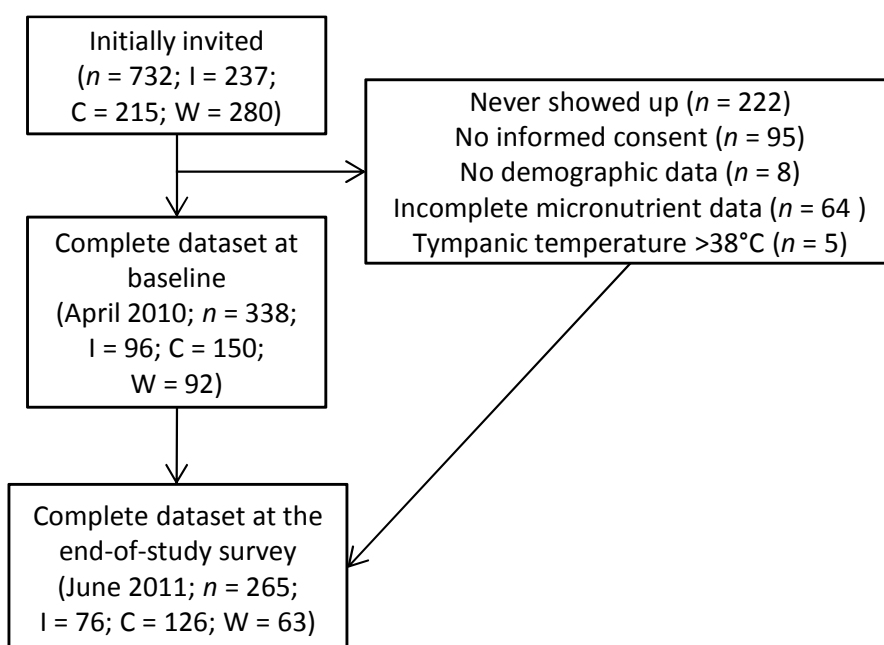


Figure 6.2: Participation's flow diagram at the baseline and the end-of-study surveys of the prospective longitudinal monitoring of anaemia carried out in three population cohorts in the Taabo health demographic surveillance system, in south-central Côte d'Ivoire.

6.4 Results

6.4.1 Biochemical characteristics of the participants

At baseline, similar numbers of males and females were enrolled in the infants and school-aged children groups (**Table 6.1**). The prevalence of anaemia, ID, and inflammation was highest among infants, irrespective of the choice of the biomarker of iron status (sTfR or PF) or inflammation (AGP or CRP), school-aged children had the highest prevalence of *P. falciparum* infection. Women had BMIs within the normal range (18.5-25). Infants had median sTfR concentrations higher than the cut-off values used for defining ID (8.3 or 8.5 mg/L). Median PF concentrations were within the normal range in the three age groups.

Table 6.1: Demographic and biochemical characteristics of the study population in south-central Côte d'Ivoire at the baseline cross-sectional survey carried out in April 2010 (n = 338).

Characteristic	Infants (n = 96)	School-aged children (n = 150)	Women (n = 92)
Age (y)	15.1 ± 5.3 ^{1,2}	7.1 ± 0.7	19.5 ± 3.2
Female sex (n (%))	50 (52.1)	68 (45.3)	92 (100)
HAZ	-1.8 (-2.8, -1.0) ³	-0.8 (-1.6, 0.2)	NA
WAZ	-0.8 (-1.8, 0.0)	-1.0 (-1.6, -0.2)	NA
BMI (kg/m ²)	NA	NA	21.3 (20.0, 23.2)
sTfR (mg/L)	11.5 (8.5, 20.2)	7.9 (6.5, 9.7)	6.9 (5.5, 9.5)
Elevated sTfR, >8.5 (n (%))	71 (74.0)	63 (42.0)	31 (33.7)
Elevated sTfR, >8.3 (n (%))	74 (77.1)	65 (43.3)	33 (35.9)
PF (µg/L)	36.1 (16.7, 70.3)	63.1 (43.7, 104.9)	44.9 (28.3, 71.8)
Low PF, <12 (infants), <15 (n (%))	14 (15.0)	4 (2.7)	9 (9.8)
Low PF, <30 (n (%))	38 (39.6)	14 (9.3)	26 (28.3)
CRP (mg/L)	2.5 (0.7, 13.7)	1.1 (0.5, 4.3)	0.8 (0.3, 2.5)
Elevated CRP, >5 (n (%))	36 (37.5)	32 (21.3)	12 (13.0)
AGP (g/L)	1.1 (0.9, 1.4)	0.9 (0.8, 1.1)	0.8 (0.6, 0.9)
Elevated AGP, >1 (n (%))	55 (57.3)	54 (36.0)	10 (10.9)
CRP >5 and AGP >1 (n (%))	35 (36.5)	27 (18.0)	4 (4.4)
Haemoglobin (g/L)	97 ± 15	116 ± 10	120 ± 15
Anaemia (n (%))	77 (80.2)	71 (47.3)	43 (46.7)
<i>P. falciparum</i> infection (n (%)) ⁴	40 (41.7)	118 (78.7)	34 (37.0)

¹ Value is in months.

² Mean ± SD (all such values).

³ Median; 25th and 75th quartiles in parentheses (all such values).

⁴ *P. falciparum* infection is defined as a positive rapid diagnostic test (RDT) or the detection of asexual *Plasmodium* parasites by microscopic examination of thick and thin blood films.

AGP, α1 acid glycoprotein; CRP, C-reactive protein; HAZ, height-for-age Z-score; N/A, not applicable; PF, plasma ferritin; sTfR, soluble transferrin receptor; WAZ weight-for-age Z-score.

The inflammatory biomarker AGP showed a significant positive correlation with both sTfR and PF in the three age groups and with *P. falciparum* parasitaemia in infants and school-aged children (**Table 6.2**). CRP showed a statistically significant positive correlation with sTfR in infants and school-aged children, and with PF concentration and *P. falciparum* in each of the three cohorts. sTfR and Hb showed significant negative correlations in all age groups, whereas PF and Hb did not significantly correlate. There was a significant positive correlation between *P. falciparum* parasitaemia and both sTfR and PF in infants and school-aged children.

Table 6.2: Spearman's correlation coefficient (ρ) between inflammatory and iron status variables in infants, school-aged children, and young women, in south-central Côte d'Ivoire at the baseline cross-sectional survey in April 2010.

Variable	Infants ($n = 96$)		School-aged children ($n = 150$)		Women ($n = 92$)	
	ρ	P value	ρ	P value	ρ	P value
sTfR - CRP	0.269	0.008	0.232	0.004	0.112	0.288
sTfR - AGP	0.373	<0.001	0.492	<0.001	0.341	<0.001
sTfR - Hb	-0.396	<0.001	-0.323	<0.001	-0.245	0.019
sTfR - <i>Pf</i> parasitaemia	0.240	0.019	0.256	0.002	0.026	0.803
PF - CRP	0.499	<0.001	0.382	<0.001	0.301	0.004
PF - AGP	0.505	<0.001	0.418	<0.001	0.355	<0.001
PF - Hb	-0.169	0.099	-0.112	0.171	0.195	0.062
PF - <i>Pf</i> parasitaemia	0.495	<0.001	0.267	0.001	0.185	0.077
PF - sTfR	-0.123	0.234	0.212	0.009	-0.109	0.300
<i>Pf</i> parasitaemia - CRP	0.465	<0.001	0.411	<0.001	0.227	0.030
<i>Pf</i> parasitaemia - AGP	0.415	<0.001	0.224	0.006	0.182	0.082
<i>Pf</i> parasitaemia - Hb	-0.410	<0.001	-0.247	0.002	-0.064	0.546

AGP, α_1 -acid glycoprotein; CRP, C-reactive protein; Hb, haemoglobin; *Pf*, *Plasmodium falciparum*; PF, plasma ferritin; sTfR soluble transferrin receptor.

In multivariate logistic regression analyses, we found no significant association between sTfR and CRP in any of the three age groups investigated (**Tables 6.3 and 6.4**). At baseline, we found a significant positive association between AGP and sTfR in infants and school-aged children after adjusting for age, sex, Hb, *P. falciparum* parasitaemia, and PF.

Table 6.3: Multivariate regression analysis with sTfR as outcome and CRP as inflammatory marker.

	Baseline cross-sectional survey, April 2010						End-of-study cross-sectional survey, June 2011					
	Infants (<i>n</i> = 96)		School-aged children (<i>n</i> = 150)		Women (<i>n</i> = 92)		Infants (<i>n</i> = 76)		School-aged children (<i>n</i> = 126)		Women (<i>n</i> = 63)	
	Coeff	<i>P</i> value	Coeff	<i>P</i> value	Coeff	<i>P</i> value	Coeff	<i>P</i> value	Coeff	<i>P</i> value	Coeff	<i>P</i> value
Age ¹	-0.29	0.172	0.15	0.626	-0.07	0.623	-0.15	0.219	-0.2	0.513	-0.06	0.716
Sex	-0.84	0.69	0.08	0.877	N/A	N/A	-1.35	0.267	0.46	0.363	N/A	N/A
Hb	-3.62	<0.001	-0.99	<0.001	-0.96	0.002	-1.46	0.003	-0.51	0.024	-0.9	0.029
Parasitaemia	-0.09	0.804	0.08	0.321	0.02	0.873	-0.11	0.589	-0.07	0.402	-0.35	0.028
CRP	0.19	0.136	-0.05	0.222	-0.04	0.635	-0.11	0.099	-0.03	0.526	-0.01	0.117
Ferritin	-0.06	0.051	0.01	0.122	0.01	0.511	0.02	0.223	0.01	0.369	-0.02	0.889
Intercept	57.77	<0.001	17.80	<0.001	20.71	<0.001	30.87	<0.001	15.48	<0.001	22.86	0.136

¹ Age refer to mo for infants and to y for school-aged children and women.
CRP, C-reactive protein; Hb, haemoglobin; N/A, not applicable; sTfR, soluble transferrin receptor.

Table 6.4: Multivariate regression analysis with sTfR as outcome and AGP as inflammatory marker.

	Baseline cross-sectional survey, April 2010						End-of-study cross-sectional survey, June 2011					
	Infants (<i>n</i> = 96)		School-aged children (<i>n</i> = 150)		Women (<i>n</i> = 92)		Infants (<i>n</i> = 76)		School-aged children (<i>n</i> = 126)		Women (<i>n</i> = 63)	
	Coeff	<i>P</i> value	Coeff	<i>P</i> value	Coeff	<i>P</i> value	Coeff	<i>P</i> value	Coeff	<i>P</i> value	Coeff	<i>P</i> value
Age ¹	-0.35	0.077	0.07	0.803	-0.04	0.797	-0.08	0.507	-0.05	0.866	-0.03	0.855
Sex	-2.43	0.215	0.09	0.861	N/A	N/A	-1.51	0.002	-0.22	0.309	N/A	N/A
Hb	-3.27	<0.001	-0.54	0.018	-0.83	0.006	-0.89	0.461	0.36	0.45	-0.87	0.029
Parasitaemia	-0.09	0.775	0.05	0.56	-0.03	0.852	-0.24	0.258	-0.13	0.103	-0.37	0.093
AGP	15.23	<0.001	5.37	<0.001	4.06	0.069	3.68	0.134	4.53	<0.001	3.26	0.118
Ferritin	-0.09	0.002	0.00	0.692	-0.01	0.678	0.00	0.743	-0.01	0.393	-0.03	0.033
Intercept	41.57	<0.001	9.29	0.013	16.03	0.002	26.57	<0.001	7.71	0.055	19.82	0.002

¹ Age refer to mo for infants and to y for school-aged children and women.
AGP, α 1 acid glycoprotein; Hb, haemoglobin; N/A, not applicable; sTfR, soluble transferrin receptor.

6.4.2 sTfR and PF ratios in infants, school-aged children and women with or without inflammation and with or without *P. falciparum* infection

At baseline, the respective percentage of subjects within the incubation, early and late convalescence stages were 1%, 36% and 21% in infants, 3%, 18% and 18% in school-aged children, and 9%, 4% and 7% in women, respectively (**Table 6.5**). sTfR and PF showed distinct profiles during incubation, early convalescence, and late convalescence (**Tables 6.5 and 6.6**). Whereas the highest concentrations of sTfR were measured at late stage of convalescence for school-aged children and women, PF concentration was highest in subjects at early convalescence. At baseline, infants, school-aged children, and women with elevated CRP and AGP (early convalescence) had significantly higher sTfR and PF concentrations than control individuals without inflammation, although this association lacked statistical significance for sTfR in women, presumably because of the low number of women with inflammation (Table 6.5). There was no significant difference in sTfR/ferritin index between participants with inflammation (incubation, early or late convalescence) and control individuals, except for school-aged children in late convalescence stage. We found similar ratios for sTfR and PF at the end-of-study survey, although for infants and women they were nonsignificant (**Table 6.6**). Infants and school-aged children with inflammation (defined as CRP >5 mg/L or AGP >1 g/L) or with *P. falciparum* infection had significantly higher sTfR concentrations than did the control individuals without inflammation, whereas the PF concentration was not affected by inflammation in the absence of parasitaemia in these two age groups (**Table 6.7**). The mean age of infants with inflammation was higher than the mean age of infants without inflammation (16.1 ± 0.6 mo *versus* 13.7 ± 0.9 mo; Wilcoxon rank sum test's p-value: 0.028). Infants with *P. falciparum* infection (with or without inflammation) were significantly older than infants free of infection (16.5 ± 0.7 mo *versus* 14.1 ± 0.7 mo; Wilcoxon rank sum test's p-value: 0.029). No significant difference was found in school-aged children and women.

Table 6.5: Estimation of CFs based on ratios of iron status indicators by inflammatory status in Ivorian infants, school-aged children and women at baseline (April 2010).

Infants (n = 96)				
sTfR, mg/L	n (%)	Concentration¹	Ratio (95% CI)²	CF³
Control	40 (42)	11.36 ± 8.89	N/A	N/A
Incubation	1 (1)	13.05 ± 0	1.15 (-, -)	0.87
Early convalescence	35 (36)	15.08 ± 11.07	1.33 (1.02, 1.72)	0.75
Late convalescence	20 (21)	14.48 ± 16.31	1.27 (0.96, 1.70)	0.78
PF, µg/L				
Control	40 (42)	22.78 ± 22.83	NA	NA
Incubation	1 (1)	9.30 ± 0	0.40 (-, -)	2.45
Early convalescence	35 (36)	59.80 ± 55.93	2.62 (1.82, 3.78)	0.38
Late convalescence	20 (21)	38.59 ± 44.59	1.69 (1.07, 2.69)	0.59
sTfR/ferritin index⁴				
Control	40 (42)	8.65 ± 9.64	NA	NA
Incubation	1 (1)	13.47 ± 0	1.56 (-, -)	0.64
Early convalescence	35 (36)	8.68 ± 10.77	1.00 (0.72, 1.40)	1.00
Late convalescence	20 (21)	9.49 ± 9.31	1.10 (0.76, 1.58)	0.91
School-aged children (n = 150)				
sTfR, mg/L	n (%)	Concentration¹	Ratio (95% CI)²	CF³
Control	91 (61)	7.29 ± 2.47	NA	NA
Incubation	5 (3)	6.32 ± 1.71	0.87 (0.51, 1.47)	1.15
Early convalescence	27 (18)	9.26 ± 3.93	1.27 (1.00, 1.61)	0.79
Late convalescence	27 (18)	9.80 ± 4.13	1.34 (1.06, 1.70)	0.74
PF, µg/L				
Control	91 (61)	54.73 ± 37.23	NA	NA
Incubation	5 (3)	67.97 ± 34.96	1.24 (0.73, 2.12)	0.81
Early convalescence	27 (18)	98.13 ± 44.82	1.79 (1.40, 2.29)	0.56
Late convalescence	27 (18)	67.02 ± 38.83	1.22 (0.94, 1.59)	0.82
sTfR/ferritin index⁴				
Control	91 (61)	4.25 ± 1.52	NA	NA
Incubation	5 (3)	3.47 ± 1.06	0.82 (0.62, 1.08)	1.22
Early convalescence	27 (18)	4.67 ± 2.64	1.10 (0.96, 1.27)	0.91
Late convalescence	27 (18)	5.44 ± 2.52	1.28 (1.11, 1.48)	0.78
Women (n = 92)				
sTfR, mg/L	n (%)	Concentration¹	Ratio (95% CI)²	CF³
Control	74 (80)	7.16 ± 3.79	NA	NA
Incubation	8 (9)	8.20 ± 6.83	1.14 (0.84, 1.57)	0.87
Early convalescence	4 (4)	8.06 ± 3.61	1.12 (0.75, 1.69)	0.89
Late convalescence	6 (7)	9.42 ± 5.44	1.32 (0.93, 1.85)	0.76
PF, µg/L				
Control	74 (80)	37.30 ± 30.14	NA	NA
Incubation	8 (9)	59.83 ± 51.37	1.60 (0.92, 2.79)	0.62
Early convalescence	4 (4)	139.09 ± 56.02	3.73 (1.76, 7.87)	0.27
Late convalescence	6 (7)	51.94 ± 42.61	1.39 (0.74, 2.61)	0.72
sTfR/ferritin index⁴				
Control	74 (80)	4.67 ± 3.77	NA	NA
Incubation	8 (9)	4.71 ± 3.49	1.01 (0.68, 1.45)	0.99
Early convalescence	4 (4)	3.77 ± 1.43	0.80 (0.48, 1.35)	1.25
Late convalescence	6 (7)	5.58 ± 3.89	1.48 (0.77, 1.85)	0.68

¹ Values are geometric mean ± SDs; ² Geometric mean ratio that compares the iron biomarker concentration at each inflammatory stage with that of the control group; ³ Estimated from the ratios of the geometric means as explained in references 22 and 33; ⁴ Calculated as sTfR (mg/L)/log PF (µg/L). CF, correction factor; NA, not applicable; PF, plasma ferritin; sTfR, soluble transferrin receptor

Table 6.6: Estimation of CFs using ratios of iron status indicator geometric means by inflammatory status in Ivorian infants, school-aged children and young women at the end-of-study survey (June 2011).

Infants (n = 76)				
sTfR, mg/L	n (%)	Concentration¹	Ratio (95% CI)²	CF³
Control	31 (41)	8.36 ± 4.18	NA	NA
Incubation	2 (3)	6.43 ± 6.62	0.77 (0.39, 1.53)	1.3
Early convalescence	15 (20)	10.70 ± 8.67	1.28 (0.95, 1.72)	0.78
Late convalescence	28 (37)	9.84 ± 3.74	1.18 (0.96, 1.45)	0.85
PF, µg/L				
Control	31 (41)	36.07 ± 33.92	NA	NA
Incubation	2 (3)	33.44 ± 37.66	0.93 (0.32, 2.73)	1.08
Early convalescence	15 (20)	96.32 ± 75.11	2.67 (1.59, 4.48)	0.37
Late convalescence	28 (37)	50.63 ± 45.91	1.40 (0.92, 2.13)	0.71
sTfR/ferritin index⁴				
Control	31 (41)	5.49 ± 4.06	NA	NA
Incubation	2 (3)	4.32 ± 6.15	0.79 (0.32, 1.90)	1.27
Early convalescence	15 (20)	5.59 ± 5.81	1.02 (0.70, 1.48)	0.98
Late convalescence	28 (37)	5.95 ± 3.66	1.08 (0.82, 1.43)	0.92
School-aged children (n = 126)				
sTfR, mg/L	n (%)	Concentration¹	Ratio (95% CI)²	CF³
Control	76 (60)	6.94 ± 2.61	NA	NA
Incubation	7 (6)	6.65 ± 3.35	0.96 (0.59, 1.55)	1.04
Early convalescence	16 (13)	7.80 ± 2.13	1.12 (0.82, 1.54)	0.89
Late convalescence	27 (21)	8.96 ± 3.17	1.29 (1.01, 1.66)	0.77
PF, µg/L				
Control	76 (60)	61.16 ± 41.59	NA	NA
Incubation	7 (6)	73.82 ± 81.60	1.21 (0.75, 1.95)	0.83
Early convalescence	16 (13)	116.24 ± 130.21	1.90 (1.36, 2.66)	0.53
Late convalescence	27 (21)	77.96 ± 31.38	1.27 (0.99, 1.64)	0.78
sTfR/ferritin index⁴				
Control	76 (60)	3.93 ± 2.48	NA	NA
Incubation	7 (6)	3.58 ± 1.73	0.91 (0.67, 1.24)	1.10
Early convalescence	16 (13)	3.81 ± 1.30	0.97 (0.79, 1.19)	1.03
Late convalescence	27 (21)	4.76 ± 1.64	1.21 (1.02, 1.43)	0.83
Women (n = 63)				
sTfR, mg/L	n (%)	Concentration¹	Ratio (95% CI)²	CF³
Control	50 (79)	7.08 ± 4.05	NA	NA
Incubation	5 (8)	7.59 ± 6.15	1.07 (0.71, 1.63)	0.93
Early convalescence	3 (5)	6.81 ± 2.13	0.96 (0.58, 1.59)	1.04
Late convalescence	5 (8)	9.08 ± 5.56	1.28 (0.85, 1.93)	0.78
PF, µg/L				
Control	50 (79)	39.70 ± 31.64	NA	NA
Incubation	5 (8)	49.75 ± 54.84	1.25 (0.62, 2.55)	0.80
Early convalescence	3 (5)	83.44 ± 78.45	2.10 (0.86, 5.13)	0.48
Late convalescence	5 (8)	49.49 ± 32.55	1.25 (0.62, 2.49)	0.80
sTfR/ferritin index⁴				
Control	50 (79)	4.54 ± 4.66	NA	NA
Incubation	5 (8)	4.56 ± 4.30	1.01 (0.58, 1.74)	0.99
Early convalescence	3 (5)	3.58 ± 1.70	0.79 (0.40, 1.55)	1.27
Late convalescence	5 (8)	5.40 ± 3.77	1.19 (0.70, 2.03)	0.84

¹ Values are geometric mean ± SDs; ² Geometric mean ratio that compares the iron biomarker concentration at each inflammatory stage with that of the control group; ³ Estimated from the ratios of the geometric mean as

explained in references 22 and 33; ⁴ Calculated as sTfR (mg/L)/log PF (µg/L). CF, correction factor; NA, not applicable; PF, plasma ferritin; sTfR, soluble transferrin receptor.

Table 6.7: Estimation of CFs using ratios of iron status indicator geometric means by infection status in Ivorian infants, school-aged children and young women at baseline (April 2010).

Infants (n = 96)				
sTfR, mg/L	n (%)	Concentration¹	Ratio (95% CI)²	CF³
Control	33 (34)	10.28 ± 8.09	NA	NA
Inflammation alone	23 (24)	14.85 ± 17.68	1.45 (1.04, 2.01)	0.69
<i>Pf</i> infection alone	7 (7)	18.17 ± 9.87	1.77 (1.15, 2.72)	0.57
<i>Pf</i> infection and inflammation	33 (34)	13.80 ± 8.36	1.44 (1.13, 1.84)	0.69
PF, µg/L				
Control	33 (34)	22.68 ± 20.28	NA	NA
Inflammation alone	23 (24)	27.12 ± 34.35	1.20 (0.77, 1.85)	0.84
<i>Pf</i> infection alone	7 (7)	23.28 ± 30.69	1.03 (0.53, 1.98)	0.97
<i>Pf</i> infection and inflammation	33 (34)	75.20 ± 53.15	3.32 (2.33, 4.72)	0.30
sTfR/ferritin index⁴				
Control	33 (34)	7.83 ± 9.91	NA	NA
Inflammation alone	23 (24)	10.75 ± 13.78	1.37 (0.91, 2.07)	0.73
<i>Pf</i> infection alone	7 (7)	13.84 ± 7.52	1.77 (1.00, 3.11)	0.57
<i>Pf</i> infection and inflammation	33 (34)	8.00 ± 5.53	1.02 (0.75, 1.39)	0.98
School-aged children (n = 150)				
sTfR, mg/L	n (%)	Concentration¹	Ratio (95% CI)²	CF³
Control	26 (17)	6.54 ± 1.90	NA	NA
Inflammation alone	6 (4)	10.06 ± 5.88	1.54 (1.17, 2.03)	0.65
<i>Pf</i> infection alone	65 (43)	7.62 ± 2.60	1.16 (1.02, 1.34)	0.86
<i>Pf</i> infection and inflammation	53 (35)	9.10 ± 3.77	1.39 (1.20, 1.62)	0.72
PF, µg/L				
Control	26 (17)	50.94 ± 39.15	NA	NA
Inflammation alone	6 (4)	45.31 ± 46.65	0.89 (0.46, 1.71)	1.12
<i>Pf</i> infection alone	65 (43)	56.32 ± 36.67	1.11 (0.84, 1.45)	0.90
<i>Pf</i> infection and inflammation	53 (35)	85.19 ± 42.59	1.67 (1.30, 2.15)	0.60
sTfR/ferritin index⁴				
Control	26 (17)	3.88 ± 0.94	NA	NA
Inflammation alone	6 (4)	6.31 ± 3.91	1.63 (1.23, 2.15)	0.61
<i>Pf</i> infection alone	65 (43)	4.40 ± 1.66	1.13 (0.98, 1.31)	0.88
<i>Pf</i> infection and inflammation	53 (35)	4.74 ± 2.31	1.22 (1.04, 1.43)	0.82
Women (n = 92)				
sTfR, mg/L	n (%)	Concentration¹	Ratio (95% CI)²	CF³
Control	52 (57)	7.05 ± 3.92	NA	NA
Inflammation alone	6 (7)	8.83 ± 4.84	1.25 (0.87, 1.80)	0.80
<i>Pf</i> infection alone	22 (24)	7.45 ± 3.55	1.06 (0.86, 1.29)	0.95
<i>Pf</i> infection and inflammation	12 (13)	8.42 ± 6.08	1.20 (0.90, 1.58)	0.84
PF, µg/L				
Control	52(57)	36.08 ± 29.09	NA	NA
Inflammation alone	6 (7)	50.49 ± 46.02	1.40 (0.73, 2.69)	0.71
<i>Pf</i> infection alone	22 (24)	40.43 ± 32.93	1.12 (0.77, 1.63)	0.89
<i>Pf</i> infection and inflammation	12 (13)	80.39 ± 60.68	2.23 (0.70, 1.80)	0.45
sTfR/ferritin index⁴				
Control	52 (57)	4.64 ± 3.97	NA	NA
Inflammation alone	6 (7)	5.30 ± 4.20	1.14 (0.72, 1.81)	0.88
<i>Pf</i> infection alone	22 (24)	4.75 ± 3.35	1.02 (0.79, 1.33)	0.98
<i>Pf</i> infection and inflammation	12 (13)	4.49 ± 2.82	0.97 (0.69, 1.35)	1.03

¹ Values are geometric means ± SDs; ² Geometric mean ratio that compares the iron biomarker concentration at each infection stage with that of the reference group; ³ Estimated from the ratios of the geometric mean as explained in

references 22 and 33; ⁴ Calculated as sTfR (mg/L)/log ferritin (µg/L). CF, correction factor; N/A, not applicable; *Pf*, *Plasmodium falciparum*; PF, plasma ferritin; sTfR, soluble transferrin receptor

6.4.3 Adjustment of sTfR and PF concentrations and of sTfR/ferritin index for inflammation or *P. falciparum* infection

At baseline, correction of sTfR for inflammation only, or for inflammation and *P. falciparum* infection in infants significantly decreased the estimated prevalence of ID from 74% to 60% and 54%, respectively, whereas correction of PF for inflammation significantly increased ID prevalence from 15% to 23% (**Table 6.8**). In school-aged children, adjustment of sTfR or the sTfR/ferritin index for inflammation or *P. falciparum* infection significantly decreased the estimated prevalence of ID, whereas adjustment of PF for inflammation had no effect. In women, correction of sTfR or PF for *P. falciparum* infection and inflammation significantly decreased or, respectively, increased the prevalence of ID. The prevalence of ID in infants and school-aged children was lower at the end-of-study survey than at baseline. In these two cohorts, correction of sTfR for inflammation had similar effects at the end-of-study survey than at baseline (**Table 6.9**).

Table 6.8: Effect of correcting soluble transferrin receptor (sTfR) and plasma ferritin (PF) concentrations on the prevalence of iron deficiency in infants (6-23 months), school-aged children (6-8 years), and young women (15-25 years) at baseline (April 2010).

Indicators	Infants with ID (<i>n</i> = 96)		School-aged children with ID (<i>n</i> = 150)		Women with ID (<i>n</i> = 92)	
	<i>n</i> (%)	<i>P</i> value ¹	<i>n</i> (%)	<i>P</i> value ¹	<i>n</i> (%)	<i>P</i> value ¹
sTfR >8.5 mg/L						
Uncorrected	71 (74.0)	NA	63 (42.0)	NA	31 (33.7)	NA
Corrected for both CRP and AGP	58 (60.4)	<0.001	40 (26.7)	<0.001	30 (32.6)	0.317
Corrected for CRP only	58 (60.4)	<0.001	60 (40.0)	0.083	31 (33.7)	N/A
Corrected for AGP only	57 (59.4)	<0.001	39 (26.0)	<0.001	30 (32.6)	0.317
Corrected for <i>Plasmodium</i> and inflammation	52 (54.2)	<0.001	21 (14.0)	<0.001	27 (29.4)	0.046
No inflammation	23 (57.5)	NA	28 (30.8)	NA	23 (31.1)	NA
PF <12 (infants), <15 g/L						
Uncorrected	14 (14.6)	NA	4 (2.7)	NA	9 (9.8)	NA
Corrected for both CRP and AGP	22 (22.9)	0.011	4 (2.7)	NA	12 (13.0)	0.083
Corrected for CRP only	20 (20.8)	0.014	4 (2.7)	NA	11 (12.0)	0.157
Corrected for AGP only	24 (25.0)	0.002	4 (2.7)	NA	10 (10.9)	0.317
Corrected for <i>Plasmodium</i> and inflammation	22 (22.9)	0.005	4 (2.7)	NA	14 (15.2)	0.025
Corrected for CF Thurnham ²	20 (20.8)	0.014	5 (3.3)	0.317	12 (13.0)	0.083
No inflammation	10 (25.0)	NA	3 (3.3)	NA	9 (12.2)	NA
sTfR/ferritin index³						
Uncorrected	70 (72.9)	NA	41 (27.3)	NA	33 (35.9)	NA
Corrected for both CRP and AGP	69 (71.9)	0.317	31 (20.7)	0.004	35 (38.0)	0.157
Corrected for CRP only	70 (72.9)	1.000	42 (28.0)	0.317	33 (35.9)	1.000
Corrected for AGP only	68 (70.8)	0.157	30 (20.0)	0.001	33 (35.9)	1.000
Corrected for <i>Plasmodium</i> and inflammation	65 (67.7)	0.025	21 (14.0)	<0.001	33 (35.9)	1.000
No inflammation	29 (72.5)	NA	21 (23.1)	NA	25 (33.8)	NA

¹ Mc Nemar's chi square of proportion was used to compare estimate prevalence from uncorrected and corrected values; ² CFs obtained from a meta-analysis by Thurnham et al. (Thurnham et al., 2010). Infants: incubation 0.88, early convalescence 0.48, late convalescence 0.70; school-aged children: incubation 0.64, early convalescence 0.39, late convalescence

0.65; women: incubation 0.73, early convalescence 0.58, late convalescence 0.85; ³ Calculated as sTfR (mg/L)/log ferritin (µg/L). AGP, α1 acid glycoprotein; CRP, C-reactive protein; Hb, haemoglobin; ID, iron deficiency; N/A, not applicable; PF, plasma ferritin; sTfR, soluble transferrin receptor.

Table 6.9: Effect of correcting soluble transferrin receptor (sTfR) and plasma ferritin (PF) concentrations on the prevalence of iron deficiency (ID) in infants (6-23 mo), school-aged children (6-8 y), and women (15-25 y) at the end-of-study survey (June 2011).

Indicators	Infants with ID (<i>n</i> = 76)		School-aged children with ID (<i>n</i> = 126)		Women with ID (<i>n</i> = 63)	
	<i>n</i> (%)	<i>P</i> value ¹	<i>n</i> (%)	<i>P</i> value ¹	<i>n</i> (%)	<i>P</i> value ¹
sTfR >8.5 mg/L						
Uncorrected	43 (56.6)	NA	47 (37.3)	NA	21 (33.3)	NA
Corrected for both CRP and AGP	32 (42.1)	0.001	36 (28.6)	0.003	20 (31.8)	0.317
Corrected for CRP only	43 (56.6)	1.000	47 (37.3)	1.000	21 (33.3)	1.000
Corrected for AGP only	32 (42.1)	0.001	36 (28.6)	0.003	19 (30.2)	0.317
Corrected for <i>Plasmodium</i> and inflammation	31 (40.8)	<0.001	37 (29.4)	0.008	20 (31.8)	0.317
No inflammation	13 (41.9)	NA	23 (30.3)	NA	15 (30.0)	NA
PF <12 (infants), <15 g/L						
Uncorrected	6 (7.9)	NA	2 (1.6)	NA	8 (12.7)	NA
Corrected for both CRP and AGP	8 (10.5)	0.317	3 (2.4)	0.317	9 (14.3)	0.317
Corrected for CRP only	7 (9.2)	0.317	3 (2.4)	0.317	9 (14.3)	0.317
Corrected for AGP only	9 (11.8)	0.157	3 (2.4)	0.317	8 (12.7)	1.000
Corrected for <i>Plasmodium</i> and inflammation	7 (9.2)	0.317	3 (2.4)	0.317	9 (14.3)	0.317
Corrected for CF Thurnham ²	8 (10.5)	0.317	3 (2.4)	0.317	9 (14.3)	0.317
No inflammation	2 (6.5)	NA	2 (2.6)	NA	8 (16.0)	NA
sTfR/ferritin index³						
Uncorrected	36 (47.4)	NA	27 (21.4)	NA	21(33.3)	NA
Corrected for both CRP and AGP	32 (42.1)	0.046	20 (15.9)	0.014	21 (33.3)	1.000
Corrected for CRP only	36 (47.4)	1.000	28 (22.2)	0.317	21(33.3)	1.000
Corrected for AGP only	32 (42.1)	0.046	22 (17.5)	0.046	21 (33.3)	1.000
Corrected for <i>Plasmodium</i> and inflammation	37 (48.7)	0.564	27 (21.4)	1.000	28 (44.4)	0.083
No inflammation	14 (45.2)	NA	11 (14.5)	NA	16 (32.0)	NA

¹ Mc Nemar's chi square of proportion to compare estimate prevalence from uncorrected and corrected values; ² CFs obtained from a meta-analysis by Thurnham et al. (Thurnham et al., 2010). Infants: incubation 0.88, early convalescence 0.48, late convalescence 0.70; school-aged children: incubation 0.64, early convalescence 0.39, late convalescence 0.65; women: incubation 0.73, early convalescence 0.58, late convalescence 0.85; ³ Calculated as sTfR (mg/L)/log ferritin (µg/L). AGP, α1 acid glycoprotein; CRP, C-reactive protein; Hb, haemoglobin; ID, iron deficiency; N/A, not applicable; PF, plasma ferritin; sTfR, soluble transferrin receptor.

6.5 Discussion

Data from this 14-mo prospective longitudinal monitoring study in 3 different age groups in a primarily rural part of Côte d'Ivoire showed that both sTfR and PF concentrations can be influenced by inflammation and *P. falciparum* infection status. Although infants and school-age children with inflammation but free of *P. falciparum* infection had significantly higher sTfR concentrations than did their counterparts without inflammation, inflammation without parasitaemia did not significantly alter PF concentrations. Importantly, we showed that adjustment of baseline sTfR values for inflammation resulted in a considerable decrease in the estimated prevalence of ID from 74% to 59% in infants and from 42% to 26% in school-age children. Our findings are important because sTfR has previously been considered not to be affected by inflammation, and sTfR is increasingly being used to measure the prevalence of ID in settings where inflammatory disorders are widespread and/or malaria is endemic (Staubli-Asobayire et al., 2001; Cercamondi et al., 2010; Shinoda et al., 2012; Grant et al., 2012).

Our study has several limitations. First, the sample size was relatively small, and hence did not allow comparison of CFs for the three inflammation groups between subjects with and without *P. falciparum* infection. Furthermore, the overall low prevalence of inflammation in women (~20%) might have blunted a significant association between inflammation and iron status biomarkers in this age group. Second, the mean age of infants with inflammation or with *P. falciparum* infection was slightly higher than the mean age of their counterparts without inflammation or without *P. falciparum* infection. However, as sTfR concentration decreased with the age of the infants (overall Spearman's ρ : -0.24, $p = 0.001$) it is not conceivable that the significantly higher sTfR concentrations observed in infants with inflammation or *P. falciparum* infection were explained by higher age in this group. Third, considering that our study did not specifically investigate the causes of inflammation due to factors other than infection with *P. falciparum*, we cannot exclude that other infectious diseases (e.g., HIV/AIDS and diarrhoea) trigger sTfR productions in response to haemolysis or ineffective erythropoiesis. A general limitation encountered in field studies in resource-constrained settings is the unfeasibility of collecting bone marrow aspirate, which in turn prevents to compare the specificity and sensitivity of sTfR and PF in estimating ID prevalence with this unambiguous and objective measure. Another limitation found in most studies using AGP as inflammatory marker is the lack of an objective cut-off defining inflammation. As an example, in this study the prevalence of ID in infants significantly increased from 35% (95% confidence interval (CI) 26-45%) to 62% (95% CI 51-71%) based

on sTfR concentrations adjusted for inflammation defined as AGP >0.7 g/L or AGP >1.1 g/L, respectively.

Considering the inflammatory stages used by Thurnham et al. (Thurnham et al., 2010) and others (Grant et al., 2011), sTfR and PF showed distinct dynamics in response to inflammation. The relative number of infants in the different phases of convalescence was similar to what has been observed in Kenya and Pakistan in subjects with similar age ranges (Paracha et al., 2000; Grant et al., 2011). The profiles of sTfR and PF during the different stages of convalescence suggest that they are differentially regulated by inflammation. Whereas PF reacts promptly and markedly to inflammation, the concentrations of sTfR are highest during the early and late stages of convalescence. On the one hand, the sTfR concentration might increase relatively to TfR expression in erythroid progenitor cells, in response to the lack of iron for erythropoiesis. The lack of plasma iron results from an increased in the hepcidin concentration, which prevents iron – exported either from macrophages or from enterocytes – from entering the plasma (Beguin, 2003). On the other hand, TfR transcription, and hence sTfR concentration, might be stimulated in macrophages in response to increased hypoxia-inducible factor-1 (HIF-1) translation due to infection and/or inflammation (Dery et al., 2005; Tacchini et al., 2008). Previous studies have shown that HIF-1 binds to the promoter site of TfR gene, which might increase TfR transcription (Lok and Ponka, 1999). The regulation of sTfR, and of CRP and AGP, by inflammatory cytokines is not well characterised; thus, new research is needed to deepen the understanding of sTfR regulation in response to inflammation. The observation that inflammation status influenced sTfR concentrations slightly less after 14 mo of selective medical interventions, coupled with a significantly lower prevalence of anemia in the infants and school-age children groups, suggests that ID might also contribute to the difference in sTfR concentration between individuals with and without subclinical inflammation.

The observation that PF and inflammatory biomarkers were significantly positively correlated in the three cohorts studied here confirms the widely acknowledged poor sensitivity of this marker to detect ID in populations with high prevalence of infections or inflammations (Kalantar-Zadeh et al., 2006). Furthermore, our data confirm the results of two recent studies conducted among Kenyan and Zimbabwean preschool-age children, which reported a significant association between inflammation status and sTfR concentration (Kasvosve et al., 2006; Grant et al., 2011). The observation that infants and school-aged children with *Plasmodium* infection have significantly higher concentrations of sTfR also confirm previous findings (Menendez et al., 2001; Verhoef et al., 2001) and suggest that, in this age group, malaria-induced haemolysis is coupled with an increased erythropoietic rate. The lack of a significant difference in sTfR concentration between women with and without inflammation

might be explained by the relatively small sample size in this age group. Yet, our findings might indicate that the consequence of inflammation differs across age classes. Of note, two infants (7 and 10 mo of age) with $\geq 5,000$ *Plasmodium* parasites/ μL of blood and tympanic temperature $>38^\circ\text{C}$ (excluded for the analyses presented here) had sTfR concentration <1 mg/L, which indicates that clinical malaria may decrease sTfR concentration (Beesley et al., 2000).

In relation to a correction for the elevated PF, the current study indicates that the use of the CFs obtained in a previous meta-analysis (Thurnham et al., 2010) provide similar results (Table 6.8). However, the use of CFs per se might be questionable, because we found considerable differences between the estimated prevalence of ID based on sTfR or PF concentrations, either uncorrected or corrected for inflammation. Other studies have found a large difference in ID prevalence between estimates based on sTfR and PF in infants and school-age children (Staubli-Asobayire et al., 2001; Cardoso et al., 2012; Grant et al., 2012). In relation to elevated sTfR/ferritin indexes, because our results indicate that both ID and inflammation are associated with higher sTfR concentrations, the utility of this marker to distinguish subjects with anaemia of inflammation from subjects with anaemia of inflammation and ID might be challenged (Suominen et al., 1998; Malope et al., 2001). Indeed, it is likely that the sTfR/ferritin index overlaps considerably between individuals with anaemia of inflammation only and those with anaemia of inflammation and ID anaemia; hence, this would prevent the use of this index to distinguish between both types of anaemia.

In conclusion, our findings indicate that the sTfR concentration is higher in infants and school-aged children with subclinical inflammation or with *P. falciparum* infection. Moreover, ID prevalence is significantly lower when CFs based on sTfR concentrations in otherwise comparable subjects with no inflammation are applied. As reported elsewhere, PF concentration was significantly higher in infants and school-aged children affected by subclinical inflammation and ID prevalence increased when CFs were applied. However, because ID prevalence estimated based on sTfR or PF (with or without corrections) showed considerable differences, caution is still needed when measuring iron status in areas of widespread inflammatory disorders or high endemicity of malaria. Our observations should be considered when setting up refined guidelines about the prevention and control of ID in malaria-endemic countries (Harding et al., 2012). Indeed, if screening for ID is maintained as a pre-requisite to iron supplementation (Crowley et al., 2012), accurate and robust markers must be used; therefore, caution is currently indicated if sTfR or PF is being used.

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7. Local concepts of anaemia-related illnesses and their public health implications in the Taabo health demographic surveillance system, Côte d'Ivoire

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7.1 *Abstract*

Background: A 14-month prospective longitudinal study conducted in the Taabo health demographic surveillance system (HDSS), south-central Côte d'Ivoire, revealed high prevalence of anaemia in different population groups in three types of settings (i.e., small town, village, and hamlet). Demographic parameters and several variables related to parasitic infections, micronutrient status, and inflammation were significantly associated with higher odds of anaemia. However, cultural concepts and knowledge of various anaemia-related illnesses and their relation with people's behaviours have not been investigated.

Methods: Sixteen focus group discussions and six key informant interviews were performed with village authorities, health workers, and traditional healers. Questionnaires were administered to 200 school-aged children and 115 young women. Of these individuals, 206 participated in the preceding longitudinal study, whereas the remaining 109 people were not exposed to prior research, but had similar age and sex profiles. Mean prominence of participants' responses was compared between groups of participants and across study settings.

Results: Local concepts of anaemia-related illnesses referred to its perceived causes based on two logical frameworks - biomedical and sociocultural - although a clear distinction was often blurred. We found few differences in knowledge, beliefs, and behaviours across study settings and between participants who were exposed to prior research and newly recruited ones. Malaria and nutritional issues as understood and managed by the population differed from definitions and recommendations provided by the health system. Malaria was not acknowledged as an exclusive mosquito-transmitted disease and participants referred to the quantity, rather than the quality, of food when talking about nutritional issues.

Conclusions: Local concepts and ideas about anaemia have public health implications, inasmuch as they are related to people's attitudes, risk-related and help-seeking behaviours, which in turn might affect their health status. Local terminology and beliefs about anaemia and malaria should be carefully considered when developing health intervention and education programs. The similarity in knowledge about anaemia-related illnesses and associated behaviours, regardless of study setting and prior exposure to research, suggests that a uniform communication strategy may be used to develop education programmes and awareness campaigns aimed at the prevention and control of anaemia in south-central Côte d'Ivoire.

7.2 Background

Anaemia, a term referring to a reduction in the number of red blood cells (RBC), haemoglobin (Hb) concentration, or oxygen-binding capacity of Hb, affects all population groups. Indeed, an estimated 2 billion people suffer from this condition worldwide (McLean et al., 2009). Pregnant women and young children and, more generally, the poorest of the poor who live in settings where malnutrition and infectious diseases are widespread are most affected (WHO, 2000; McLean et al., 2009). In the humid tropics, anaemia is multifactorial with malaria, iron deficiency (ID), and helminth infections among the most important contributing factors to low Hb levels (Crawley, 2004; Tolentino and Friedman, 2007; Soares Magalhães and Clements, 2011).

A situation analysis is the first step in developing education and intervention programs. Such an analysis not only entails defining the epidemiology or the extent of a given condition, but also requires assessing the health and economic status of residents, and study of people's perceptions and attitudes toward the disease and potential interventions. Knowledge and related behaviours might vary depending on many parameters (e.g. demographic, sociologic and economic parameters, genders, exposure to prior research or programs, seasons, etc.) (Winch et al., 1994; Tanner and Vlassoff, 1998; Babu et al., 2001).

In a preceding 14-month prospective, longitudinal study carried out in the Taabo health demographic surveillance system (Taabo HDSS) in south-central Côte d'Ivoire, which commenced in April 2010, we investigated the association between biomedical variables and anaemia among infants (6-23 months), school-aged children (6-8 years), and young women (15-25 years) from three different settings. We found that, depending on age groups, *Plasmodium falciparum* and *Schistosoma haematobium* infections, inflammation, cellular iron deficiency, and chronic malnutrition were significantly and positively linked to the prevalence of anaemia in this area (Righetti et al., 2012; Righetti et al., 2013).

Previous questionnaire-based studies pursued in sub-Saharan Africa primarily focused on knowledge, attitudes, practices, and beliefs (KAPB) among specific population groups, emphasizing single aetiological agent of anaemia, such as malaria (11-13) (Beiersmann et al. 2007; Esse et al., 2008; Ouattara et al., 2011), soil-transmitted helminth infections (Acka et al., 2010), ID (Galloway et al., 2002), or sickle-cell trait (Wonkam et al., 2006). Some other studies have investigated local concepts related to malaria (Ahorlu et al., 2005; Beiersmann et al., 2007). An ethnographic study carried out in Abidjan highlighted the implications of community understandings in the prevention and control of malaria (Granado et al., 2011). However, basic concepts of blood and various anaemia-related illnesses, the perception of

the multifactorial aetiology of anaemia and their public health implications have yet to be characterised.

The purpose of this study was to deepen our knowledge of local concepts of blood and anaemia in three settings of the Taabo HDSS. The specific objectives were (i) to define local concepts related to blood and anaemia; (ii) to investigate the relationship between these concepts and local health problems; and (iii) to assess the heterogeneity of this relationship throughout the study area. Potential implications for public health are discussed placing emphasis on how findings affect risk-related and help-seeking behaviours.

7.3 *Materials and methods*

7.3.1 Ethical considerations

The study protocol was approved by the institutional research commissions of the Swiss Tropical and Public Health Institute (Swiss TPH; Basel, Switzerland, reference no. FK 96). Ethical approval was granted by the ethics committee of Basel (EKBB, reference no. 252/09) and Côte d'Ivoire (reference no. 1086 MSHP/CNER). Village chiefs, participants, and parents/guardians of children were informed about the purpose and procedures of the study. Written informed consent (or fingerprints of illiterate people and minors) was obtained from all interviewed participants and the parents/guardians of children <16 years of age.

7.3.2 Study area and design

The study was conducted in the Taabo HDSS, situated in south-central Côte d'Ivoire. Taabo Cité, the only small town within the Taabo HDSS is located some 160 km north-west of Abidjan, the economic capital, and some 60 km south of Yamoussoukro, the political capital of Côte d'Ivoire. Taabo HDSS is part of the Agnéby-Tiassa region, one of the 30 new administrative regions of Côte d'Ivoire designated in September 2011 by the new President of Côte d'Ivoire. The study area lies in the V-Baoulé, a transition zone from rainforest in the South to Savannah in the North, more precisely in the Eburean climatic area. There are four seasons: (i) a long rainy season lasting from April to July, (ii) a short dry season in the months of August and September, (iii) a short rainy season in October and November, and (iv) and a long dry season, lasting from December to March. There are many small valleys in an overall flat landscape. The ferralitic soils are favourable to many types of cultivations found in forest zones (e.g., cacao, cassava, coffee, and rubber) and in Savannah areas (e.g., cassava and yam). The large hydroelectric dam, built in the late 1970s on the Bandama River, is a central feature of the area (N'Goran et al., 1997).

The design of our study was a cross-sectional survey, using a mixed methods approach (qualitative and quantitative), done in February 2012. The study was integrated into a larger project with the overarching goal to deepen our understanding of the aetiology of anaemia in the Taabo HDSS and to investigate potential preventive and control measures (Righetti et al., 2012b). Preceding the current work, from April 2010 to June 2011, we monitored anaemia longitudinally in three age groups (infants, school-aged children, and women) in three settings of the Taabo HDSS (Ahondo, one of 14 villages, Katchénou a former hamlet, and Taabo Cité). The three study cohorts were selected for their high risk of anaemia or the high prevalence of parasitic infections, or both. The sample size of the three study cohorts was calculated based on the assumed prevalence of anaemia in the Taabo area (Righetti et al., 2012b). We found that several variables were significantly associated with anaemia, including parasitic, nutritional, inflammatory, and demographic variables (Righetti et al., 2012a; Righetti et al., 2012b).

The current KAPB survey aimed at furthering our understanding of the local cultural knowledge and beliefs about anaemia and their relation with people's behaviours. Participants were selected according to the following criteria. First, we invited all school-aged children and women who had participated in one of the two last cross-sectional surveys of the previous longitudinal study (n=206). Additionally, we recruited 109 individuals who had not been exposed to prior research about anaemia, but were otherwise comparable to the participants of the 14-month prospective longitudinal monitoring in terms of age, sex, and setting. Overall, the study was conducted in five localities that were representative of the three main types of settlement found in the Taabo area, namely (i) small town, (ii) village, and (iii) hamlet (i.e., small village that usually lacks social structures such as health facility and school, and is not officially registered as a village in the national registry). This sampling frame allowed us to minimize selection bias and, although not a main objective of this study, gave us the opportunity to investigate whether the longitudinal monitoring had an influence on people's knowledge and beliefs about anaemia.

Taken together, our study was conducted in (i) Taabo Cité, a small town with approximately 7,000 inhabitants, a district hospital with 12 beds staffed by a medical doctor and a surgeon, where both participants and non-participants to the longitudinal monitoring were enrolled; (ii) Ahondo, a village located in close proximity to Lake Taabo where all children (aged 6-8 years at baseline) and women (aged 15-25 years at baseline) were exposed to prior research and where there is a health dispensary with a nurse; (iii) Sahoua, a neighbouring village of Ahondo where people have not been exposed to the longitudinal study; (iv) Katchénou, a former hamlet, enrolled in the longitudinal survey, where there was no health dispensaries at the time of the study; and (v) Amani Kouadiokro, a neighbouring and very similar hamlet of

Katchénou with no prior research. Depending on meteorological conditions, the population of both hamlets can access the health dispensary of Sokrogbo, located some 5 km away.

7.3.3 Characteristics of target and main ethnic groups

Study respondents were school-aged children (8-10 years) and young women of reproductive age (17-27 years). According to the Taabo HDSS database, in February 2012, when the current study was carried out, 40,574 people were registered as permanent residents in the Taabo HDSS.

The construction of a hydroelectric dam on the Bandama River in the late 1970s attracted people from different parts of Côte d'Ivoire and neighbouring countries to Taabo, and hence the population has become quite cosmopolitan. Indigenous people are members of the Akan (mainly Baoulé) and the Krou ethnic groups (Bété), two of the four large groups of Côte d'Ivoire. Migrants from other regions of Côte d'Ivoire include people from the four ethnic groups, Akan (other Baoulé, Abidji, Agni, Akyé, Alladjan, etc.), Krou (Dida, etc.), Gur (Koulango, Senoufo, etc.), and South and North Mandé (South Mandé: Yacouba, Gouro, etc.; North Mandé: Malinké, Dioula, Kôyaka, etc.). Migrants from other nations mainly come from other African countries, especially from Burkina Faso, Mali, and Nigeria.

7.3.4 Study instruments

Both qualitative and quantitative methods were used to gather information about anaemia-related illnesses from school-aged children and young women, as well as health workers, traditional healers, and other village authorities. First, an information meeting was organized with all community chiefs from Taabo Cité in order to inform and mobilize the communities about the upcoming survey. During this meeting, local terms for blood and anaemia were gathered. Second, questionnaires were pre-tested, and administered to women and school-aged children to assess KAPB about blood and various anaemia-related illnesses for quantitative analysis. The questionnaires were divided in six parts: (i) sociodemographic parameters; (ii) local concepts and knowledge of blood and anaemia-related illnesses; (iii) experience of anaemia-related illnesses; (iv) prevention of anaemia-related illnesses; (v) reported treatment-seeking behaviour; and (vi) 24-hour food recall and nutritional habits. Results from our previous epidemiological study and literature review informed the development of our questionnaire and the categories for responses. The prominence of coded categories was based on whether responses identifying that category were reported spontaneously in response to an open question, only in response to probing for that category, or not reported at all. If a category was spontaneously reported, this gave a prominence of 2. If the category was only reported after probing, this gave a prominence of

1. A category not reported received prominence 0. This grading system allows calculating a mean prominence for each category. Multiple responses were permitted. The second author (AAR) coded spontaneously reported responses with reference to the categories of probed questions. The percentage of reporting in each category spontaneously and after having been probed is reported in the Tables.

Questionnaires were pre-tested in two steps. First, five questionnaires were administered by the first author (MKDK) to check if children and women could understand the questions, to determine whether to add probed questions and to refine categories. For instance, the question “what would you do if you have anaemia?” was not posed to children as the pre-test showed that they were unable to respond without asking for the parent’s opinion.

Second, four field enumerators were trained to administer questionnaires. Each enumerator administered at least one questionnaire to a child and a woman in French and in Baoulé, under the supervision of two of us (MKDK and AAR). This training was continued until each enumerator was at ease with the questionnaire. Field workers were fluent in French, Baoulé, and Dioula, the three main local languages. In the rare cases that a participant did not understand any of these languages, a third person helped with translation.

Focus group discussions (FGDs) were conducted by the first author (MKDK) in French or Baoulé and tape recorded by the second author (AAR). In each locality, FGDs were conducted with children aged 8-10 years, women aged 17-27 years, village authorities, and, in Taabo Cité, the medical staff of Taabo General Hospital to collect in-depth information about specific questions (**Table 7.1**). Groups included between 8 and 12 people. Key informant semi-structured interviews were conducted with traditional healers and the nurse of the health dispensary in Ahondo. In one hamlet (Amani Kouadiokro), no traditional healer was recognized by the whole population. Qualitative data were analysed by the first author who participated in all interviews, made notes in writing from the tape-record version and extracted relevant information for the results presented here.

Table 7.1: Number of key informant interviews, focus group discussions, and questionnaires carried out in the five study localities of the Taabo health demographic surveillance system, south-central Côte d'Ivoire in February 2012.

Locality	Key informant interviews		Focus group discussions				Questionnaires	
	Health staff	Traditional health practitioner	School-aged children	Women	Village authorities	Health staff	Children	Women
Ahondo (village)	1	1	1	1	1	0	40	39
Amani-Kouadiokro (hamlet)	0	0	1	1	1	0	19	15
Katchénou (hamlet)	0	1	1	1	1	0	45	14
Sahoua (village)	0	1	1	1	1	0	13	22
Taabo Cité (town)	0	2	1	1	1	1	83	25
Total	1	5	5	5	5	1	200	115

7.3.5 Statistical analysis

Questionnaire data were entered twice in Microsoft Access version 10.0 (2007 Microsoft Corporation). Double-entered datasets were compared using EpiInfo version 3.4.1 (Centers for Disease Control and Prevention; Atlanta, GA, USA), and discrepancies were removed by going back to the original questionnaire. Data were analysed using Stata version 10 (StataCorp; College Station, TX, USA).

Household socioeconomic status was calculated using an asset-based index (Filmer and Pritchett 2001). Data on household assets (e.g., possession of a radio), housing characteristics (e.g., walls constructed with bricks), and the number of people per room were obtained from the existing Taabo HDSS database. Using principal component analysis (PCA) to weight the binary data of these variables, we subsequently divided the households into three socioeconomic groups (wealth tertiles): (i) very poor; (ii) poor; and (iii) least poor. Food was categorized according to indicators put forward by the World Health Organization (WHO) (WHO 2008). The percentage of reporting in each category spontaneously and after having been probed is reported in tables. Responses from children and women are reported separately to easily visualize potential differences between the two age groups and because the administered questionnaires were slightly different. The mean prominence of reported variables (2, spontaneously reported; 1, probed; 0, not reported) was compared across study settings (town, village, hamlet) and between individuals who have been exposed to prior research and newly recruited participants, using the Kruskal Wallis test and Wilcoxon rank-sum test, respectively. Significance was set as a p-value adjusted for ties <0.05.

7.4 Results

7.4.1 Socioeconomic characteristics of the study population

Table 7.2 shows that the socioeconomic status of participants differed among study setting. Whilst more than 60% of women and school-aged children residing in Taabo Cité belong to the least poor tertile, more than 90% of the participants living in hamlets belong to the poorest tertile of the population. Moreover, while 60.0% of the women and 95.2% of the children living in Taabo Cité attended school, the respective proportions were considerably lower in rural areas. In Taabo Cité, retailing is the main activity of 40.0% of women, whereas farming is the principal occupation of 86.2% of the women interviewed in hamlets. The villages of Ahondo and Sahoua represent an intermediate situation between town and hamlet for all parameters.

Table 7.2: Socioeconomic characteristics of the study population, stratified by setting and age group.

Characteristics	Town (N = 108)		Village (N = 114)		Hamlet (N = 93)	
	Women n (%)	Children n (%)	Women n (%)	Children n (%)	Women n (%)	Children n (%)
Socio-demographic indicator						
Poorest	2 (8.0)	2 (2.4)	9 (14.8)	7 (13.0)	28 (96.6)	59 (90.8)
Poor	7 (28.0)	30 (36.1)	30 (49.2)	27 (50.0)	1 (3.5)	6 (9.2)
Least poor	16 (64.0)	51 (61.5)	22 (36.1)	20 (37.0)	0	0
Mean age (years)	20	9	21.6	8.9	22.2	8.8
Female	25 (100.0)	38 (45.8)	61 (100.0)	19 (35.9)	29 (100.0)	31 (48.4)
Principal language of the interview						
French	21 (84.0)	66 (79.5)	28 (45.9)	14 (26.4)	4 (13.8)	3 (4.7)
Other	4 (16.0)	17 (20.5)	33 (54.1)	39 (73.6)	25 (86.2)	58 (95.3)
Went to school	15 (60.0)	79 (95.2)	21 (34.4)	43 (81.1)	6 (20.7)	49 (76.6)
Can read and write	12 (48.0)	73 (87.6)	15 (24.6)	32 (60.4)	3 (10.3)	26 (40.6)
Occupation						
Farmer	1 (4.0)	N/A	33 (54.1)	N/A	25 (86.2)	N/A
Merchant	10 (40.0)	N/A	18 (29.5)	N/A	1 (3.5)	N/A
Housekeeper	6 (24.0)	N/A	8 (13.1)	N/A	1 (3.5)	N/A
Student	4 (16.0)	N/A	2 (3.3)	N/A	1 (3.5)	N/A
Other	4 (16.0)	N/A	0	N/A	1 (3.5)	N/A

N/A, not applicable

7.4.2 Local terms and representations of blood and anaemia-related

Local terms reported by community chiefs for blood and anaemia-related illnesses, and their approximate English translation are summarized in **Table 7.3**. Whilst the word “blood” did exist in each local language with no difference in meaning, there was no direct translation for “anaemia”. The local terms used to describe anaemia-related illnesses indicate how these conditions are understood by the population.

A representation is the constructed image, the meaning or the association people make with another element or condition. **Table 7.4** summarizes the representations of blood and anaemia-related illnesses among women and school-aged children. The representations of blood included four main categories: “something which is in the body”, “life”, “strength”, and “health”. Data from the FGDs elaborate these concepts:

“Blood makes the body work. Man is born with blood. If you don’t have blood, this means you are not a man. Blood is like the motor of man” (community chiefs in Taabo Cité).

Table 7.3: Local terms for blood and anaemia and their approximate translation into English in south-central Côte d'Ivoire.

Ethnic groups	Language groups	Local terms for blood	Local terms for anaemia	Conceptual translation of local terms for anaemia
Akan	Abidji	<i>mbouo</i>	<i>mbouo ohou</i>	Blood is over
	Agni	<i>modja</i>	<i>modja wa wié</i>	Blood is over
	Akyé	<i>vûn</i>	<i>opou vûn</i>	Blood is over
			<i>o vûn ésè</i>	Blood decreased
	Alladjan	<i>inkrè</i>	<i>inkrè tro</i>	Blood is over
	Baoulé	<i>modja</i>	<i>modja wa vié</i>	Blood is over
<i>modja wa kpéssou</i>			Blood decreased	
<i>modja djouman</i>			There is insufficient blood	
Krou	Bété	<i>drou</i>	<i>drou yé bia</i>	Blood is over
Gur (voltaic)	Koulango	<i>tôm</i>	<i>tôm bayô</i>	Blood is lacking
			<i>tôm la</i>	Blood is over
	Senoufo	<i>chichan</i>	<i>chichan N'kwô</i>	Blood is over
			<i>chichan yôrôgo</i>	Blood decreased
Tagwana	<i>dissiant</i>	<i>dissiant wo manni</i>	Blood is lacking	
North Mandé	Malinké	<i>bassi ; djoli</i>	<i>bassi banan</i>	Blood is over
			<i>bassi dôgô yala</i>	Blood decreased
South Mandé	Yaouba	<i>gno; abèr</i>	<i>gnon gnin,</i> <i>abèr yagnin</i>	Blood is over Blood is over
	Gouro	<i>gnin</i>	<i>è gnan tara</i>	Blood is over

Table 7.4: Local representations of blood and anaemia among women and children, across study settings.

Characteristics	Town (N = 108)		Village (N = 114)		Hamlet (N = 93)	
	Women <i>n (%)</i>	Children <i>n (%)</i>	Women <i>n (%)</i>	Children <i>n (%)</i>	Women <i>n (%)</i>	Children <i>n (%)</i>
Representation of blood						
In the body	7 (28.0)	47 (56.6)	6 (9.8)	28 (52.8)	4 (13.8)	19 (29.7)
Life	15 (60.0)	4 (4.8)	20 (32.8)	2 (3.8)	8 (27.6)	4 (6.3)
Health	1 (4.0)	1 (1.2)	4 (6.6)	2 (3.8)	4 (13.8)	6 (9.4)
Strength	0	1 (1.2)	22 (36.1)	1 (2.3)	9 (31.0)	11 (18.3)
Other	4 (16.0)	26 (31.3)	11 (18.0)	14 (26.4)	7 (24.1)	14 (21.9)
Do not know	0	4 (4.8)	1 (1.6)	7 (13.2)	0	16 (25.0)
Representation of anaemia-related illnesses						
Death	14 (56.0)	58 (69.9)	21 (34.4)	25 (47.2)	10 (34.5)	43 (67.2)
Illness	10 (40.0)	15 (18.1)	37 (60.7)	16 (30.2)	16 (55.2)	12 (18.8)
Weakness	1 (4.0)	1 (1.2)	1 (1.6)	1 (1.9)	1 (3.5)	2 (3.1)
Other	0	5 (6.7)	5 (8.2)	4 (9.1)	1 (3.5)	4 (6.7)
Do not know	0	9 (10.8)	0	9 (17.0)	1 (3.5)	4 (6.3)

Spontaneous responses are reported. Multiple responses were permitted.

The 25% of children who suggest other ideas about blood typically explain, “blood is red water” or “blood is *bissap*” (a locally produced ice tea from hibiscus calyx). Anaemia-related illnesses were mainly associated with either “death” or “illness”. Other representations of these conditions suggest that “this is bad stuff”, and “you cannot walk, you cannot do anything”.

7.4.3 Relationship between anaemia-related illnesses and local health problems

Figure 7.1 shows that participants identify various causes of anaemia. Spontaneously, *djékouadjo* (malaria-like illnesses) is the cause reported most often, both by school-aged children and women. However, FGDs showed that people’s ideas about malaria differ from biomedical definitions of health workers:

“*Djékouadjo* might be caused by the sun, by hard work, by mosquitoes, or by the consumption of red oil and oily food” (women in Katchénou).

Indeed, sun, hard work, oily food, and mosquitoes were the most frequently reported causes of *djékouadjo*. Key informant interviews revealed that *djékouadjo* is a term which might group together different illnesses: the symptoms of *djékouadjo yassoua* (*yassoua* = male) resemble to severe or cerebral malaria, whereas *djékouadjo bla* (*bla* = female) refers to less severe febrile illnesses, and “*djékouadjo-ôklouè*” (*ôklouè* = yellow) is a febrile illness associated to yellow-colored eyes. Furthermore, there are cultural beliefs about how malaria-like illnesses cause anaemia. In Amani Kouadiokro, for example, a woman explained that:

“The mosquito sucks your blood, and finishes it up, step by step”.

Diet, ill-health, fire, and sun are other important causes of anaemia reported by both population groups. FGDs confirmed the results obtained from our questionnaire survey, as expressed by a woman in Amani Kouadiokro:

“Diseases like *djékouadjo*, from mosquitoes and flies, hard work, too many childbirths, or sitting too often next to the fire: these are all circumstances which can finish your blood”.

Fire was overall identified as an important cause of anaemia and a traditional healer from Taabo Cité explained this causal relationship:

“When you are sitting next to the fire, the fire draws your blood”.

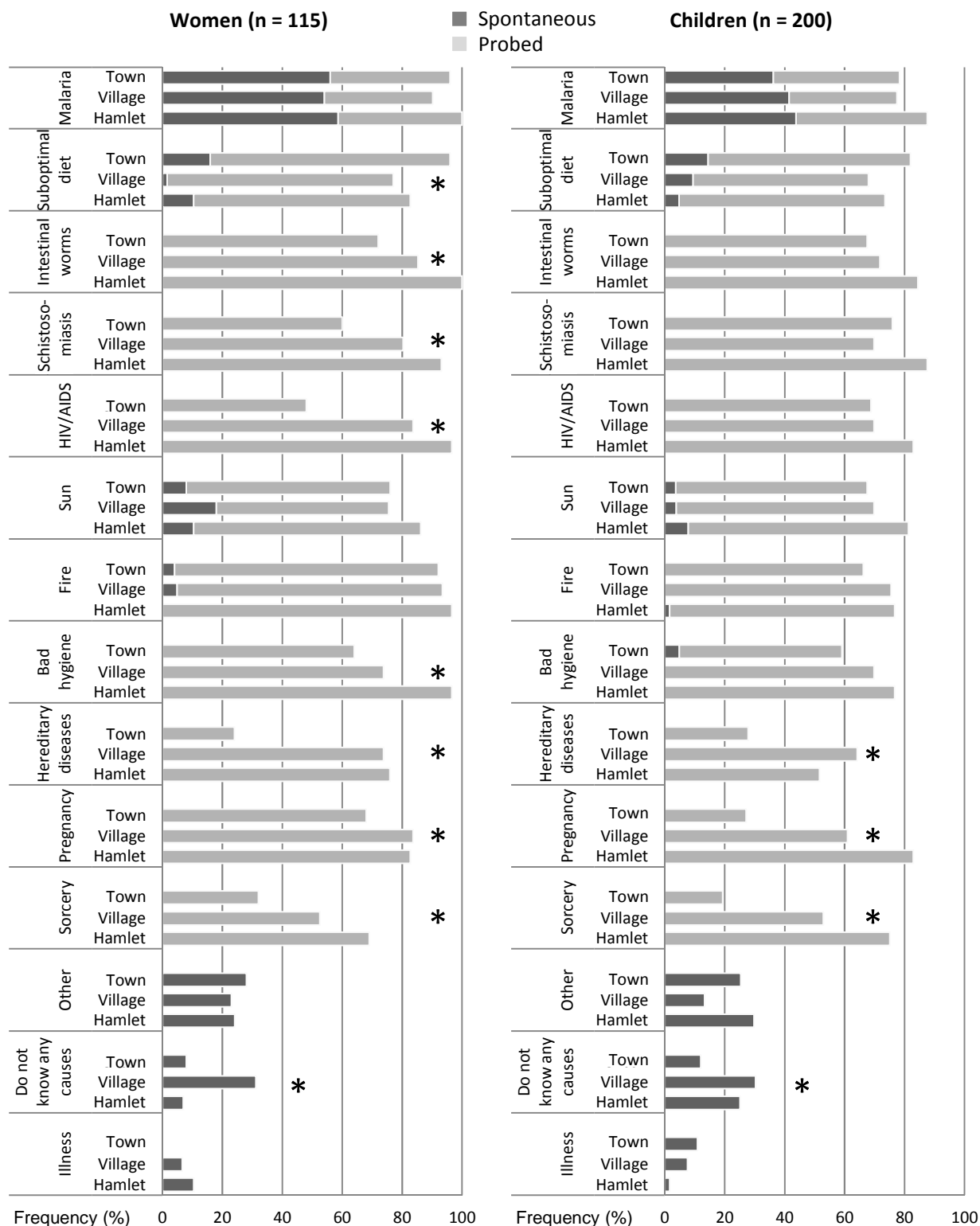


Figure 7.1: Perceived causes of anaemia among children and young women in south-central Côte d'Ivoire.

Spontaneous (dark grey) and probed (light grey) answers are reported and the mean prominence is compared between study settings with the Kruskal-Wallis test. Asterisks indicate p-value <0.05.

Other more technical considerations argue that:

“You see it when you put an animal on a fire. Blood becomes solid, coagulates and can no more circulate within the body” (communities’ chiefs in Taabo Cité).

Whilst women from rural areas more often reported schistosomiasis, intestinal worms, HIV/AIDS, and lack of hygiene as causes of anaemia, the mean prominence of suboptimal diet was significantly higher in Taabo Cité. FGDs with village authorities revealed that a couple of food plants are indeed seasonal (e.g., mangos, yams, and eggplants). However, according to village authorities, the majority of food is available throughout the year and unvaried diet is due to limited food preference and/or availability characteristic of each ethnic group. Furthermore, both children and women from rural areas reported more often that sorcery might trigger anaemia, compared with children and women living in town. A traditional healer in Taabo Cité explained:

“Sorcerers might draw your blood”.

Communities’ chiefs detailed this process as follows:

“There are two types of blood: the sweet one, which has not been prepared, and the sour one, which has been prepared with traditional medicine. Sorcerers only drink unprepared, sweet blood”.

Another recurrent cause of anaemia which emerged during FGDs was *coco* (haemorrhoids):

“Coco is not a good thing. It spoils your blood and then you get very tired” (traditional healer in Katchénou).

Participants reported three main types of symptoms associated with anaemia. They include pallor, loss of weight, and weakness/tiredness (**Figure 7.2**). The qualitative data from the FGDs clarify these findings:

“Your body is white, your eyes are white, when you eat something, you vomit it; you lose weight, you get dizzy, you are weak and your body heats up” (women in Katchénou).

Of note, mean prominence of pallor as a symptom of anaemia was significantly lower among children and women who had been exposed to prior research than among newly recruited participants.

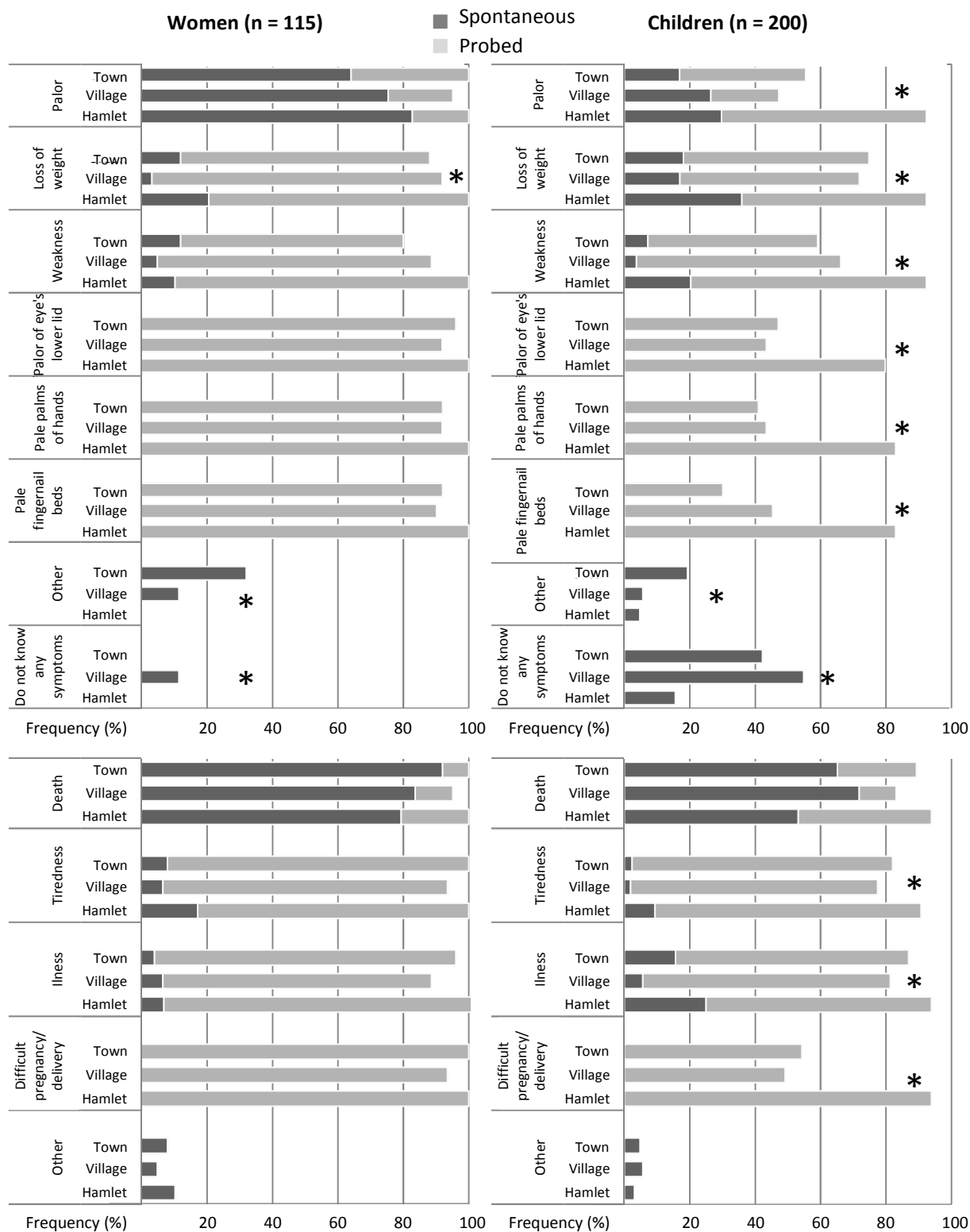


Figure 7.2: Perceived symptoms and consequences of anaemia among children and young women in south-central Côte d'Ivoire.

Spontaneous (dark grey) and probed (light grey) answers are reported and the mean prominence is compared between study settings with the Kruskal-Wallis test. Asterisks indicate p-value < 0.05.

Three main consequences of anaemia were identified among spontaneous answers from women and school-aged children: weakness, illness, and death. Death is the most frequently reported consequence of anaemia, both by women (84.4%) and children (63.0%). Four children stated that “anaemia is happiness”, “anaemia gives strength”, and “anaemia gives health”.

The two main sources of knowledge about anaemia in both population groups stem from other family members and from medical staff at health centres (data not shown). The school was mentioned as another source of knowledge, whereas no participants spontaneously reported television or radio as their source of information.

7.4.4 Help-seeking and reported behaviours for anaemia-related illnesses

Whilst women mainly reported the consumption of leafy vegetables as an effective measure to prevent anaemia (**Table 7.5**), school-aged children stated that good food, meat and fish and *bissap* are effective to prevent anaemia. Both women and school-aged children spontaneously declared that visits to the health centre in case of ill-health or during health check-ups are important to prevent anaemia. FGDs with village authorities and women emphasized that medicine and tonics are important preventive measures against anaemia:

“If you don’t want to have anaemia, you have to take medicine. There is medicine you get from the doctor but we also have leaves, here, which give you blood” (communities’ chiefs in Taabo Cité).

Other behaviours reported by more than 5% of the participants include consumption of meat, Coca-Cola, and tomatoes, as well as protective behaviours against *djékouadjo*. Prayers and spiritual practices were more often reported by individuals from rural areas whilst drinking Coca-Cola was only reported by inhabitants of Taabo Cité. During FGDs, several children reported they should be clean and behave well to prevent anaemia-related illnesses:

“You should neither play in rubbish nor in filth. You should not contradict, nor bite people” (children from Katchénou).

Most of the women interviewed told us they would go to the health centre if they were anaemic, whilst 17.3% would take traditional medicine and 6.4% put forward other curative measures (data not shown).

Table 7.5: Preventive behaviours reported against anaemia in south-central Côte d'Ivoire.

Characteristics	Women (N = 115)				Children (N = 200)			
	Spontaneous	Probed	<i>p</i> (KW)	<i>p</i> (WRS)	Spontaneous	Probed	<i>p</i> (KW)	<i>p</i> (WRS)
	<i>n</i> (%)	<i>n</i> (%)			<i>n</i> (%)	<i>n</i> (%)		
Good diet ^a	26 (22.6)	82 (71.3)	0.122	0.206	46 (23.0)	121 (60.50)	0.402	0.048
Protection from malaria ^b	15 (13.0)	90 (78.3)	0.053	0.395	15 (7.5)	158 (79.0)	0.11	0.899
Drink <i>bissap</i>	14 (12.2)	92 (80.0)	0.073	0.689	36 (18.0)	129 (64.5)	0.083	0.553
Eat meat, fish	16 (13.9)	76 (66.1)	0.046	0.191	10 (20.0)	132 (66.0)	0.448	0.058
Traditional medicine	4 (3.5)	95 (82.6)	0.417	0.641	2 (1.0)	143 (71.5)	0.005	0.267
Prayers, spiritual practices	0	65 (56.5)	0.005	0.014	5 (2.5)	124 (62.0)	<0.001	0.131
Good hygiene	3 (4.5)	0	0.443	0.707	13 (6.5)	N/A	0.075	0.983
Eat leafy vegetables	29 (25.2)	N/A	0.705	0.618	5 (2.5)	N/A	0.694	0.157
Drink Coca-Cola	9 (7.8)	N/A	0.022	0.238	13 (6.5)	N/A	0.001	0.985
Eat tomatoes	11 (9.6)	N/A	0.028	0.109	10 (5.0)	148 (74.0)	0.679	0.146
Visit health centres	32 (27.8)	76 (66.1)	0.106	0.433	34 (17.0)	132 (66.0)	0.11	0.794

Mean prominence of reported variables (coded 2 if spontaneously reported; 1 if reported after probing; and 0 if not reported) of women and children are reported. Mean prominence of reported variables have been compared across study settings and between people who had been exposed to prior research and those newly recruited with the Kruskal Wallis (KW) and Wilcoxon rank-sum (WRS) tests, respectively, and associated p-values are reported. Multiple responses were permitted.

^a Spontaneous responses include “we shall eat enough/good food/vitamins”, “we shall watch for our alimentation” (see discussion)

^b Three types of spontaneous answers are grouped here: “not walking under the sun”, “protection against *djékouadjo*” and “protection against mosquitoes”

^c People either mention “we shall do regular health check-up” or “we shall go to the doctor when we are ill”

N/A, not applicable

Table 7.6 shows that slightly more people are sleeping under LLINs in Taabo Cité than in villages and hamlets, although the difference lacked statistical significance ($\chi^2 = 3.99$, P value = 0.136). More than half of the school-aged children and women reported having eaten meat or fish the day before the interview. Twenty-four-hour food recall indicated that most participants did not consume food from three or more groups in a single meal on the day preceding the interview.

Several participants - mainly young women - acknowledged that they have had anaemia. The mean prominence of having experienced anaemia was neither significantly different across study settings, nor between individuals who have been exposed to prior research and newly recruited participants. All participants who experienced anaemia reported that they received modern medicine to treat this condition. However, not all cases of anaemia were diagnosed and treated in health centres. In addition to modern treatments, some participants received medicine from traditional healers, mainly in the hamlets (100% of women and 50% of children). Two women and one child knew they had received iron supplements and two women identified other tonics. Other children and women were unable to identify which kind of medicine they received from the health staff.

Table 7.6: Preventive and help-seeking behaviours, experience of illness and treatment use in relation to anaemia in south-central Côte d'Ivoire.

Characteristics	Town (N = 108)		Village (N = 114)		Hamlet (N = 93)	
	Women	Children	Women	Children	Women	Children
	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)
Proportion of people sleeping under LLINs ^a	0.51		0.45		0.37	
Ate food containing haeme-iron ^b	18 (72.0)	38 (50.7)	38 (65.5)	24 (54.6)	26 (89.7)	51 (85.0)
Food from ≥ 3 groups in a single meal ^b	10 (40.0)	19 (25.7)	20 (34.5)	12 (27.9)	10 (34.5)	26 (43.3)
Experienced anaemia	8 (32.0)	10 (13.3)	10 (17.2)	2 (4.6)	6 (20.7)	3 (5.0)
Anaemia diagnosed in a health centre	7 (87.6)	6 (60.0)	9 (90.0)	2 (100)	2 (33.3)	1 (33.3)
Received modern medicine	8 (100)	10 (100)	10 (100)	2 (100)	6 (100)	3 (100)
Received medicine from a traditional healer	1 (12.5)	2 (20.0)	3 (30.0)	0	3 (50.0)	3 (100)

^a The estimated number of individuals who slept under a LLIN the night preceding the interview was obtained from the Taabo HDSS database (October 2011). Hence, they are not specific to children and women but refer to the whole population of the localities.

^b 24-hour recall

7.5 Discussion

To our knowledge, this is the first study to investigate local concepts of blood and various anaemia-related illnesses among school-aged children and young women, and their potential public health implications for risk-related and help-seeking behaviours, in a multi-ethnic setting of West Africa. Using a mixed methods approach to examine quantitative and qualitative data, we found that although the biomedical term anaemia does not exist in the main local languages, the semantic form of the ancient Greek term *ἀναιμία*, meaning without blood, corresponds to the concepts used in local folk languages to report this condition. Our results reveal biomedical and sociocultural features of the knowledge of children and women for anaemia-related illnesses. These representations appear to be connected to their risk-related and help-seeking behaviours reported by children and women and to differ from professionally recognized causes of anaemia.

7.5.1 Limitations

Our study has several limitations. First, although we identified different terms related to mild and severe anaemia during the initial meeting with traditional authorities, no distinction was made between these two categories in our questionnaire survey. Such a distinction would have required very subtle terminology, particularly for school-aged children, but this was not feasible given our tight time schedule and limited human and financial resources to conduct the study. Future KAPB surveys pertaining to anaemia might investigate the implications of “relative” and “absolute” terms in relation to people’s behaviours and practices. In turn, such knowledge might provide important information for locally adapted communication strategies to prevent anaemia.

Second, our study lacks direct observational components, which might have given more credibility and weight to the findings from the questionnaire survey. Indeed, previous studies have shown that reported and observed results differ quite considerably (Stanton et al., 1987). Nevertheless, such observations would have been challenging in the case of anaemia, since cases are difficult to identify without clinical examinations. We suggest that future similar studies, particularly hospital-based surveys, might integrate an observational component.

7.5.2 Representations of blood and anaemia-related illnesses

The difference in representations of blood across study settings might be associated with the main activity of the population that differs in rural areas and in town. Whilst inhabitants of villages and hamlets are mainly engaged in subsistence farming, a considerable number of

individuals living in Taabo Cité are occupied in the tertiary sector (e.g., merchants and staff of the hospital, the school and management of the Taabo dam), or are attending high school. This observation might explain why people's conception of blood mainly refers to strength in the more rural areas, where this condition is crucial for being productive in daily agricultural activities. People use semantic constructions associated to the ill-health aspects of anaemia. These constructions are related to relative and absolute concepts of anaemia-related illnesses. Relative concepts include "blood decreased" and "blood is not enough" and these descriptions relate to non-severe forms of anaemia. This concept of anaemia-related illnesses is found in other communities of West Africa and throughout the world (Galloway et al., 2002; Beiersmann et al., 2007). "Blood is over" indicates the absolute concept of anaemia. Hence, anaemia is also considered as a severe illness and, indeed, a cause of death. Our findings reveal that anaemia is a local priority for public health.

The important differences we observed throughout the interview between spontaneously reported and probed answers might be explained by different levels of importance in the association that people construct between aetiological agents and anaemia. The large number of individuals who spontaneously reported *djékouadjo* (malaria-like illnesses) as a cause of anaemia suggests that, in the Taabo HDSS, people construct a strong relationship between anaemia and malaria-like illnesses. This association might be explained by the severity of some malarial anaemia, which forces people to seek care at the hospital. As people do not frequently visit health centres, the information they receive there might stick into their mind. In contrast, illnesses like schistosomiasis were not spontaneously reported, most likely because there are no obvious causes of anaemia for the population. However, probing schistosomiasis as a potential cause of anaemia showed that most people think this relation exists. This observation seems quite obvious by the local terminology of schistosomiasis, which refers to "the one who urinates blood". A similar explanation can be given for HIV/AIDS; although people do not spontaneously think about it as a cause of anaemia, they considerate AIDS as a blood-related disease which renders people weak. Hence, upon probing, people agree that AIDS can cause anaemia. Another factor which might explain the discrepancy between spontaneously and probed answers is, particularly among children, that they are shy or unable to give their own opinion. However, the very low proportion of participants who positively answered to absurd questions such as "Is health a consequence of anaemia?" indicates that most respondents understood the questions and gave meaningful answers.

Health centres and other family members were identified as the main sources of knowledge about anaemia, which corresponds to the sources of knowledge identified in a previous study about the use of LLINs in Côte d'Ivoire (Centre de Recherche pour de Développement 2008).

However, it is worth mentioning that health facilities are almost exclusively used as curative health structures. This may limit effective communication between the community and the health system for effective preventive medicine. Considering that, in the current study area, a household consists, on average, of eight individuals, it is not surprising that within-family communication was identified as an important source of information. Television and radio are less important sources of knowledge, inasmuch as the infrastructure has little or no support for this form of media. At the time of our study, Katchénou and Amani Kouadiokro were still not connected to the power grid. However, a few households owned a generator, which was used for various purposes, including watching television.

7.5.3 Relationship between anaemia-related illnesses and local health problems

Our results revealed that the knowledge of participants about various anaemia-related illnesses was based either on biomedical or sociocultural concepts and a clear distinction was often blurred. The biomedical dimension includes biomedical causes and preventive attitudes and reported behaviours against anaemia, as shown in previous studies (Crawley, 2004; Tolentino and Friedman, 2007; Righetti et al., 2012). The sociocultural dimension groups together beliefs, attitudes, and reported behaviours which require further in-depth investigations.

Biomedical causes include pregnancy, *djékouadjo* (malaria-like illnesses) and suboptimal diet, which is in line with our prior research in the Taabo HDSS (Righetti et al., 2012, Righetti et al., 2013) and that of others elsewhere in sub-Saharan Africa. However, FGDs revealed that *djékouadjo* is not a synonym of malaria. Such local cultural distinctions between several malaria-like illnesses were identified elsewhere in Africa and turned out to be important parameters to take into account when developing a prevention program (Winch et al., 1996). Moreover, there are cultural beliefs which provide information about how malaria-like illnesses cause anaemia. In Amani Kouadiokro, for example, a woman explained that “the mosquito sucks your blood, and finishes it up, step by step”. Anthropomorphism explanations were also observed in Burkina Faso where women reported that “the sun drinks your blood” (Galloway et al., 2002). The perceptions of food and nutrition are also complex. People talk about good food or large quantity of food rather than on iron-rich food or a diversified diet, emphasizing the divergence within the so-called “biomedical” causes as understood by the health staff and by the population.

Sociocultural causes of anaemia-related illnesses include the sun, fire, and sorcerers. The relation between sun or fire and anaemia mainly relates to the effect of heat; whilst sun

causes anaemia through sweating, fire impacts on the fluidity of blood. Interestingly, indoor biofuel smoke has been identified as a risk factor for anaemia (Mishra and Retherford 2007), and the effect of outdoor biofuel cooking on Hb levels may be worth investigating. The belief that sorcerers can cause anaemia, mainly found among rural communities in the present studies, is encountered in several communities about different diseases, including malaria-like illnesses and tuberculosis (Ahorlu et al., 2005; Haasnoot et al., 2010).

Colour was an important parameter in the diagnosis of anaemia, particularly for people living in the most rural areas. The representations of white and yellow colours as signs of ill-health are also found in the study of tuberculosis, where white cough and white body are signs of disease (Coulibaly, 2010). Although the loss, rather than the gain, of weight was associated with anaemia, people also mentioned that anaemic individuals might become bigger, specifying that “this is not good fat”, referring to swelling and oedemas.

Both children and women identified three main consequences resulting from anaemia-related illnesses: illnesses, tiredness, and death. On the one hand, most interviewees spontaneously reported death as the ultimate consequence of anaemia-related illnesses. On the other hand, tiredness and illness both impact on working capacity, productivity and financial resources, which is particularly important in subsistence farming communities, as observed here for the Taabo HDSS.

7.5.4 Help-seeking and risk-related attitudes and behaviours for anaemia-related illnesses

Knowledge and beliefs of children and women about various anaemia-related illnesses affect their attitudes and behaviours toward preventive measures against anaemia. Although *djékouadjo* was identified as an important cause of anaemia, few people mentioned the use of LLINs as an effective preventive measure against anaemia. Sun, hard work, oily food, and mosquitoes are the most frequently reported causes of *djékouadjo*. These considerations explain why few people think about sleeping under a LLIN as a preventive measure against anaemia and confirm previous observations from Côte d'Ivoire, which showed that although 73% of interviewees utilized nets to prevent nuisance from mosquitoes, only 9% thought this measure may protect them from malaria (Doannio et al., 2006). According to the Taabo HDSS database, almost half of the people are now sleeping under a net, with a slightly higher coverage in town than in rural areas, which is much higher than in mid-2008 when the Taabo HDSS was established (Noor et al., 2009; WHO, 2009). The considerable increase of LLIN coverage can be explained by a recent national distribution campaign carried out between November 2010 and July 2011 and confirms that people use LLINs without being

aware of their preventive effect against *djékouadjo*. Similar sociocultural concepts about malaria-like illnesses have been reported from other communities across the world, whose local denominations do not exactly correspond to the biomedical term malaria (Kengeya-Kayondo et al., 1994; Winch et al., 1996; Okrah et al., 2002; Launiala and Kulmala, 2006; Mbonye et al., 2006; Esse et al., 2008; Mubyazi et al., 2008).

Children and women consider food as an important issue in the prevention of anaemia. However, the recurrent expressions “eat well” and “good food” have different meanings, depending on the population group interviewed. Whilst the consumption of leafy vegetables and vitamins are sometimes reported by adults, it is more the quantity than the quality that matters to children, although the final goal is the same for all age groups: to gain strength. Beside leafy vegetables and meat, children and women reported other foodstuffs and drinks they may use to prevent anaemia: *bissap* and tomatoes were more reported in rural areas whilst Coca-Cola was exclusively quoted by people living in Taabo Cité. These fluids were also reported by traditional healers. Interestingly, the aforementioned foodstuffs and drinks, as well as local herbal teas used to prevent or treat anaemia, are all dark red-coloured. Such a relationship between red-coloured drink and foodstuffs with anaemia has been reported for other communities (Young and Ali, 2005). Our findings therefore suggest that people may build a relationship between colour and anaemia, not only for diagnostic purposes, but also for curative measures. *Bissap* may be worth further investigating as other groups of researchers reported a high content in vitamin C, one component that can improve iron absorption (Sanou et al., 2009).

Most of the women interviewed (84.4%) said they would seek care at a health centre if they suffer from anaemia and 69.2% of the respondents who experienced anaemia said they got their diagnosis from a health centre. However, health workers consistently reported that people visit health centres at a late stage of disease. In case of ill-health, people usually visit traditional healers first. FGDs corroborate these findings; in case traditional medicines do not improve the subject's health status, then he/she may visit a health centre. These observations suggest that different concepts of anaemia-related illnesses (relative *versus* absolute) may be associated with different behaviours. However, future studies should seek a clearer distinction between these concepts to investigate whether they are associated to specific behaviours and might therefore influence public health actions.

7.5.5 Public health implications of local cultural concepts and ideas about anaemia

The discrepancy between professionally recognized causes of anaemia and accompanying preventive and curative measures, and local culturally reported causes and ideas about anaemia-related illnesses have important ramifications for public health. Indeed, our results indicate that there are additional local culturally identified causes of anaemia (e.g., sun, fire, and sorcerers), and that so-called biomedical factors are understood differently, which influence the prevention and control of anaemia by the population and the health staff.

Whilst the health staff put emphasize on the quality, rather than the quantity of food for preventing anaemia, we noted a different pattern within the population. Less than 40% of the participants effectively had a balanced diet on the day before the interview. Most people reported that they have eaten meat or fish on the day preceding the interview, but this response would need to be confirmed by active food records. Indeed, in our prior epidemiological study, we found high prevalence of iron, vitamin A (in infants and children), and riboflavin deficiencies (Righetti et al., 2012b), which indicate that the local diet does not fulfil people's micronutrients requirements. Moreover, the considerable prevalence of inflammation certainly contributes to the local burden of malnutrition through preventing efficient micronutrients absorption (Righetti et al., 2012b; Righetti et al., 2013). The situation of the Taabo HDSS, located in the V-Baoulé where the rain forest meets the Savannah area offers many agricultural and fishing opportunities. FGDs with village authorities revealed that a couple of food plants are seasonal (e.g., mangos, yams, and eggplants). However, our results highlight the influence of geographical origins on diet customs. Whilst food of communities from the North (e.g., Malinké and Sénoufo) is based on cereals (i.e., maize, sorghum, millet, and rice), tubers and plantain are the main staple food of ethnic groups from the South (Wegmüller et al., 2006; Camara, 2009).

The potential public health implications of various types of *djékouadjo* are as follows. Considering that not all *djékouadjo* are thought to be transmitted by mosquitoes, this affects people's behaviour, inasmuch they do not associate LLINs to malaria prevention and referral to the health system is only done for the most severe cases of malaria and anaemia. Of note, as blood transfusions are not available in Taabo General Hospital, severe anaemia cases are referred to other larger hospitals further away. This delay in seeking prompt and effective care and the consequence on population health has been studied in different communities throughout the world (Beiersmann et al., 2007; Getahun et al., 2010; Yadav, 2010; Ayisi et al., 2011; Girma and Tesfaye, 2011). In the case of malaria, which is responsible for most cases of severe anaemia in sub-Saharan Africa, low socioeconomic status, distance to the

nearest health centre, cost of care, and perceived adverse events of modern medicine are among the key issues people put forward not to seek prompt care at health facilities (Beiersmann et al., 2007; Getahun et al., 2010; Yadav et al., 2010). The consequences include a reduced efficacy of treatment, and hence, a higher probability of complications and even death. Data from health registries at Taabo General Hospital and Ahondo health centre indicate that during the year 2011, 64/5,539 (1.2%) and 33/1,138 (2.9%) consultations, respectively, were diagnosed with severe anaemia. Mild and moderate cases of anaemia were not systematically registered, indicating that the consequences of chronic, non-severe anaemia might be underestimated by the health staff.

7.6 Conclusions

Our study identified different local concepts of anaemia, specifying two levels of severity. Children and women construct relations between these concepts and health problems. Although malaria, nutrition, and hygiene are identified as important issues affecting the quantity of blood, they do not refer to similar concepts within the health staff and the population. These findings are of public health relevance, since people's cultural ideas about anaemia are related to their risk-related and help-seeking behaviours and might, in turn, affect their health status. These findings will have to be considered when developing health programs, inasmuch a misunderstanding of biomedical terms between the health system and the community might undermine any attempted interventions. Although some differences were found between the three study settings, the overall concept, knowledge, and behaviours related to anaemia were similar, and hence a uniform strategy may be used to develop education and intervention programs to reduce the prevalence of anaemia in the Taabo HDSS and perhaps elsewhere in Côte d'Ivoire. Coupled to health and nutritional education, school canteen should be considered as 80% of children aged 8-10 years attend school. In addition, increasing the coverage of LLINs and the understanding why sleeping under a LLIN is important and health system strengthening must be further improved. Household-based education campaigns should be explored as an entry point to decrease the burden of anaemia in sub-Saharan Africa.

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8. Discussion and conclusions

This Ph. D. thesis was facilitated by an existing and productive research partnership between the Swiss Tropical and Public Health Institute (SwissTPH), the ETH Zurich, the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (CSRS) and the Université de Cocody. The thesis pursued interdisciplinary approaches, descriptive and analytical epidemiology and linked field- and laboratory work, qualitative and quantitative approaches. Taken together, the thesis advances innovation, validation and application- three main pillars of SwissTPH- in the broad field of public health. **Table 8.1** summarises the main contribution of the present PhD thesis.

Table 8.1: Main contributions of the current PhD thesis in the nexus of the Swiss TPH.

Chapter	Innovation	Validation	Application
3	Specific nutritional and parasitic variables significantly associated with anaemia were identified for each age group.	In the Taabo HDSS, anaemia is multifactorial and <i>P. falciparum</i> infection is an important contributor to low Hb concentration in infants	Retinol-binding protein might be utilized as proxy for assessing vitamin A status with a sensitivity and specificity of 55% and 94%, respectively.
4	Children co-infected with <i>Plasmodium</i> and hookworm have lower odds of anaemia than children infected with <i>Plasmodium</i> alone	The interactions between hookworm and <i>Plasmodium</i> infections are complex and depend on the age of the subjects	
5	<i>Plasmodium</i> parasitaemia, <i>S. haematobium</i> egg counts and stunting significantly predict Hb concentration in school-aged children	Iron deficiency and inflammation are significantly associated with anaemia in all age groups	
6	Inflammation, not necessarily due to an infection with <i>P. falciparum</i> , affects sTfR concentration in infants and children	Inflammation and <i>P. falciparum</i> infection affect SF concentration in infants, children and young women	Adjusting sTfR concentration for subclinical inflammation significantly decrease the estimated prevalence of ID in infants and children
7	The understanding and interpretation of the biomedical causes of anaemia (e.g. malaria and suboptimal diet) differ between the health staff and the population		Slight differences exist between urban and rural areas but the similarity in knowledge and behaviours suggest that a unique intervention strategy targeting anaemia might be applied in the whole Taabo HDSS area

The current PhD thesis was part of a Swiss National Science Foundation (SNSF)-founded project which entailed three specific objectives for investigating the aetiology of anaemia and refining adequate strategies for preventing and controlling anaemia in south-central Côte d'Ivoire. This thesis focuses on the biomedical and social aetiology of anaemia in three age groups in the Taabo HDSS, where *Plasmodium*, soil-transmitted helminth and schistosome infections are endemic (N'Goran et al., 1997; 2001; 2003; Becker et al., 2011). In this context, we were interested in the current prevalence and intensity of *Plasmodium*, soil-transmitted helminth and schistosome infections, and the prevalence and severity of micronutrient deficiencies and anaemia. Further objectives were to assess potential associations between socioeconomic, demographic, parasitic and nutritional factors to anaemia in three specific age groups and to monitor their dynamics over a 14-month prospective study. Additionally, we investigated local concepts related to anaemia, their relation with people's behaviours and discussed the implication of these findings for local public health.

The results stemming from our baseline survey confirmed that anaemia is multifactorial and that *Plasmodium* infection and ID are important parameters which affect Hb concentrations in infants in West Africa (Verhoef et al., 2002; Ayoya et al., 2011; Righetti et al., 2012b). Moreover, our study revealed an important association of inflammation and anaemia. We identified specific variables associated with *Plasmodium* infection in each age group and with hookworm infection in school-aged children and young women, and found significant association between hookworm and *Plasmodium* infection in children, whilst women with a hookworm infection had significantly lower odds of *Plasmodium* infection (Righetti et al., 2012a). Noteworthy, we showed that children aged 8 years infected with *Plasmodium* had significantly higher Hb values, lower sTfR concentrations and lower AGP concentrations (used as proxy for inflammation) if they were concomitantly infected with hookworm, suggesting a potential protective effect of hookworm infection on anaemia in school-aged children co-infected with *Plasmodium*.

To my knowledge, this study is the first attempt to longitudinally monitor anaemia and the underlying causes in three different population cohorts (infants, children and young women) over a 14 months period in a primarily rural setting of West Africa. The design of our study was a prospective longitudinal monitoring with repeated cross-sectional surveys once every 3-4 months. Of note, specific interventions (e.g. treatment of clinical malaria, helminth infection and severe anaemia) and community-based interventions (preventive chemotherapy targeting soil-transmitted helminthiases, lymphatic filariasis and schistosomiasis) (WHO, 2006; 2010) were implemented. Results from our longitudinal study indicate that, in infants and children, the prevalence of anaemia was significantly lower at the

end-of-study survey (June 2011) compared with our baseline survey (April 2010), most likely related to growing age and improved iron status in infants and lower prevalence of *P. falciparum* and helminth infection in young school-aged children (Righetti et al., 2013a). Moreover, our results show a subtle significant negative correlation between the number of eggs of *S. haematobium* and Hb in children. Importantly, our findings indicate that inflammation significantly affected sTfR concentrations. In further analyses, we could show that subclinical inflammation significantly increases sTfR concentrations in infants and school-aged children and observed a similar trend in women, and this effect was independent of *Plasmodium* infection (Righetti et al., 2013b).

The findings from our cross-sectional questionnaire survey revealed that the population of the Taabo HDSS utilizes local terminology to describe various anaemia-related illnesses (Kouadio et al., 2013). The knowledge and beliefs they have about the disease, which are very similar throughout the area of Taabo HDSS are related to their attitudes and behaviours. The discrepancy we observed between professionally recognized causes of anaemia and local culturally reported causes and ideas about anaemia is of public health relevance since a misunderstanding of health messages from the population might blunt any attempted health intervention or education programmes.

Our findings contribute important new data and insights that are of considerable importance from diagnostic, public health and control points of views and hence are discussed here. Nevertheless, our study suffers from several limitations and these will be highlighted as well in the next sections.

8.1 Epidemiology of parasitic infections and micronutrient deficiencies in infants, school-aged children and young women in the Taabo HDSS

At baseline, we found a considerable number of infections with *S. haematobium* in Ahondo (40% in school-aged children and women), with soil-transmitted helminths in Katchénou (57% in school-aged children and women) and with *P. falciparum* in the three study settings (mean prevalence: 53%). However, the prevalence and intensities of *S. haematobium* infections were much lower than what others have found in the same area 10-15 years ago (N'Goran et al., 1997; 2001; 2003). Several explanations might explain these differences. First, with regard to *S. haematobium*, our prevalence and intensity estimates are based on single urine sample examination, whilst previous investigations employed multiple urine

sampling over three to four consecutive days. Enhanced sampling efforts result in higher prevalence estimates for schistosome infections (Bergquist et al., 2009; Utzinger et al., 2011). Second, our research focussed on young school-aged children (6-8 years), while schistosome prevalence normally peak in older school-aged children or adolescents (Woolhouse, 1998; Hotez, 2006). Third, following the construction of the large hydroelectric dam on the Bandama River in the late 1970s, many research projects and control efforts have been conducted in the area. For example, children in Taabo village have been given anthelmintic drugs on a regular basis between the late 1990s and 2001 and village volunteers have been trained for presumptive treatment with praziquantel (N'Goran et al., 2001; 2003; Becker et al., 2011). Furthermore, sanitary, ecological or seasonal parameters may also have exerted a positive effect on infection prevalence and intensity (Asaolu and Ofoezie, 2003). However, data stemming from Taabo HDSS database show that, in 2010, 70% of the households still practiced open defecation, and river (or marshland) was the principal source of drinking water for 22% of the households throughout the study area. Considering only the participants to our study, in 2010, 100%, 55% and 25% of the households in Katchénou, Ahondo and Taabo Cité, respectively, practiced open defecation and 42% of the households in Ahondo obtained drinking water from the Bandama River. In any event, the findings that *S. haematobium* infection prevalence and intensity have substantially decreased in the area of Taabo are good news, particularly in view of the absolute increased number of helminth infections between the mid 1990s and 2003 throughout sub-Saharan Africa (de Silva et al., 2003). As Taabo HDSS organize, since 2010, mass drug administration with albendazole and ivermectin and local administration of praziquantel in endemic localities once a year, it is conceivable that the prevalence and intensity of helminth infection will remain at low levels for the years to come. Moreover, community-led total sanitation has been initiated in several villages of the Taabo HDSS in 2011 and the follow-up studies in mid-2012 will shed new light on the potential additive or synergic beneficial effects of such interventions on preventive chemotherapy targeting helminthiasis at the population level.

Plasmodium infections were endemic throughout the study area (mean prevalence: 53%), with highest prevalence found among school-aged children (74% at baseline). *Plasmodium falciparum* was the predominant species. Indeed all participants infected with *Plasmodium* harboured *P. falciparum*, whereas 6.5% were coinfecting with *P. malariae*. Infants showed the highest parasitaemia. Our data are similar to results from other parts of Côte d'Ivoire and suggest that malaria is still one of the primary public health concerns in this country (Girardin et al., 2004; Silue et al., 2008; Müller et al., 2011; UNICEF, 2012). The prolonged socio-political crises (Bonfoh et al., 2011) and the overall low coverage rate of proven interventions

until 2011, such as LLINs (Noor et al., 2009) might, at least partially, explain these observations. Data stemming from Taabo HDSS database indicate that, in 2012, 40% of the population of the HDSS is sleeping under LLINs, which is much higher than estimates from 2006 (Noor et al., 2009) and 2010 (unpublished data from Taabo HDSS) which indicated that only between 5% and 14% of children below the age of 5 years were sleeping under LLINs. This important increase in the number of people sleeping under LLINs may be explained by a recent national distribution of LLIN which took place in mid-2011 in the area of Taabo. Hence, it would be important to re-assess anaemia and malaria prevalence in the Taabo HDSS in 2012, to determine the effect of higher coverage rate of LLINs on *Plasmodium* prevalence and anaemia. There are no recent data for LLINs coverage at the national level for Côte d'Ivoire and hence further national surveys are needed to estimate LLINs coverage and utilization rates throughout the country.

Estimating the precise prevalence of iron, vitamin A and riboflavin deficiency in the Taabo HDSS through biomarker quantification turned out to be a challenging task since these estimates are affected by different factors. SF and sTfR produced very different estimates of iron status as did, to a lesser extent, serum retinol and RBP for vitamin A. The cut-off defining riboflavin deficiency might also be a limitation; we used the usual cut-off of EGRAC >1.4 for defining riboflavin deficiency, although, in our population, mean EGRAC was 1.43 ± 0.26 . Complementary information about people's diet should have been obtained by complementary food records in a subsample of households which have participated to the prospective longitudinal study. Of note, the PhD student who was responsible for this task left the project after only a few months in late 2010 and the food records have been reallocated to an MSc student who only started in early 2012. The data from these food records will help us to determine the differential contribution of a suboptimal diet to anaemia. Such data should bring suggestions on how to improve local diet, through education, fortification and/or supplementation.

The relative high prevalence of inflammation (defined as AGP >1g/l; 36% in April 2010 and 35% in June 2011) and its effect on vitamin A and iron status biomarkers (Thurnham et al., 2003; Thurnham et al., 2010; Grant et al., 2011) challenges the observed prevalence. In spite of these limitations, our data indicate that the prevalence of micronutrient deficiencies is relatively homogeneous throughout the study area and similar to what has been found for school-aged children in other areas of Côte d'Ivoire (Staubli-Asobayire et al., 2001; Yapi et al., 2005; Rohner et al., 2007; Rohner et al., 2010). The prevalence of ID and inflammation in school-aged children and young women were similar in April 2010 and June 2011, which suggest that people's diet and infection status do not significantly change from one year to another. However, it might be that the years 2010-2011 were not representative (due to the

socio-economic crisis) and of a longer period and further follow-ups or independent cross-sectional studies with individuals matched for age and sex might be necessary to confirm our findings.

8.2 Aetiology of anaemia in infants, school-aged children and young women in south-central Côte d'Ivoire

At baseline, we found that *P. falciparum* infection was the only variables significantly associated with anaemia in infants, whilst cellular ID was associated with higher odds of anaemia among school-aged children and women. Moreover, chronic inflammation was significantly positively associated with anaemia in children and, in women, riboflavin deficiency and working at home were the two variables that were significantly negatively associated with anaemia. The choice of infants, school-aged children and women is based on epidemiological parameters: whilst infants and young women represent the age group at highest risk for anaemia and the most vulnerable to its consequences (WHO, 2008), children at early school-aged represent an interesting group to be studied if one wishes to capture the potential effect of parasitic infections. Indeed, school-aged children are at highest of helminth infections (Anderson and May, 1985; Keiser et al., 2002; Hotez, 2006), they may represent a group with distinct characteristics and risk factors for anaemia. Moreover, children at early school-age, whose immune system is less developed compared to older children and adults, might be prone to higher morbidity (Bundy, 2006).

Our questionnaire and focus group discussions revealed other recurrent risk factors quoted by school-aged children and young women. These include climatic (sun), occupational (cooking next to the fire), behavioural (bad hygiene) and belief (sorcery) parameters. Although we did not investigate the potential biological relationship between these parameters and anaemia, these are also integrated to the global ecologic, economic and sociologic framework of anaemia within the Taabo HDSS (**Figure 8.1**).

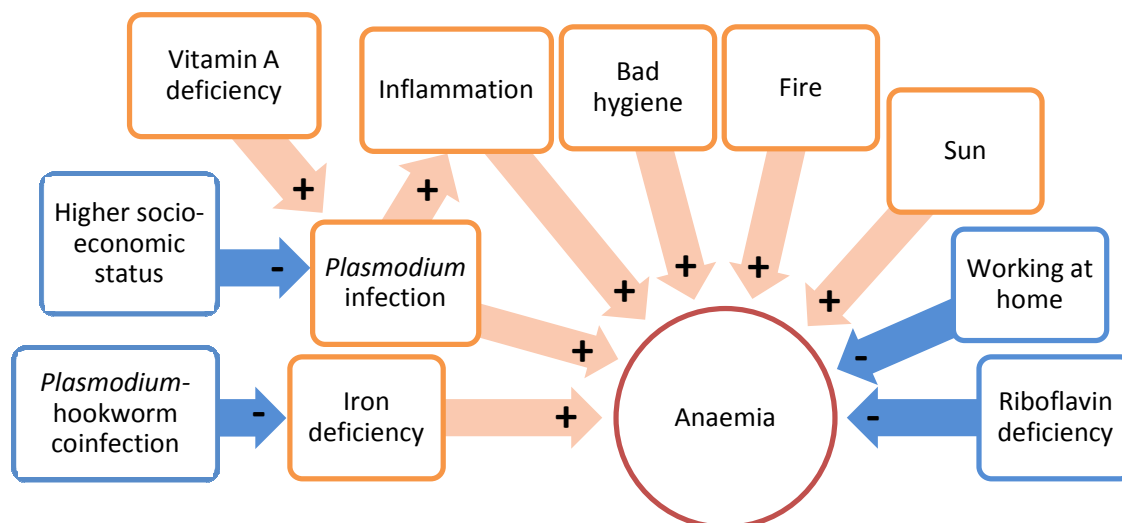


Figure 8.1: Framework of variables directly or indirectly associated with anaemia, identified by epidemiological and sociological investigations among infants, young school-aged children and young women in the Taabo HDSS in south-central Côte d'Ivoire.

Plus and minus signs represent higher and lower odds for a given outcome (e.g. *Plasmodium* infection, anaemia and inflammation), respectively. This figure highlights the principal findings of this PhD and is not exhaustive. For a global framework of anaemia as a public health problem, please refer to Figure 8.2.

8.2.1 Contributions of parasitic infections to the burden of anaemia in the Taabo HDSS

The effects of *P. falciparum* infection, including chronic asymptomatic infection and clinical malaria, on Hb concentrations in preschool-aged children have been well documented in different malaria-endemic settings across Africa (Crawley, 2004; Korenromp et al., 2004; Ronald et al., 2006; Soares Magalhães and Clements, 2011). Whilst acute clinical malaria principally triggers anaemia by haemolysis in non-immune individuals, chronic or repeated infections are more likely to cause anaemia through dyserythropoiesis (Menendez et al., 2000).

At our baseline survey, *P. falciparum* infection was the only parameter significantly associated with anaemia among infants although the prevalence of anaemia was much higher than the prevalence of *P. falciparum* infection in this age group (81% versus 44%). This observation suggests, on the one hand, that Hb cut-off defining anaemia might not be adequate for African infants. On the other hand, our relative small sample size might have blunted other significant associations. For instance, infants with storage iron depletion (defined as SF <12 µg/l in infants without inflammation and SF <30 µg/l in infants with

inflammation) showed 3.6-fold higher odds of anaemia (95% CI 0.8-15.5, adjusted for age, sex, *Plasmodium* infection, iron status and inflammation) than infants with sufficient iron stores and this association was similar in infants without *Plasmodium*, suggesting that ID also play a significant role in the development of anaemia in preschool-aged children.

In our statistical model, we integrated setting as a random factor, although our three study areas were not randomly selected within the Taabo HDSS. This is justified by the objective of identifying, in a first step, global risk factors for anaemia in representative settings for the Taabo HDSS in order to guide future local interventions that might provide important information for national level. In a second step, we computed distinct analyses for each locality. These analyses highlighted the focal distribution of schistosome infections within the study area and the related heterogeneity in risk factors distribution. Indeed, stratified analyses showed, on the one hand, that the number of eggs of *S. haematobium* was significantly negatively correlated with Hb in children living in Ahondo only. This is explained by the situation of Ahondo, located in close proximity to the Bandama River, where *S. haematobium* infection prevalence reached 40% in children and women at the beginning of the study. On the other hand, *P. falciparum* parasitaemia was significantly correlated with Hb concentrations in children living in Taabo Cité only. Children living in Taabo Cité presented lower prevalence of *Plasmodium* infection compared to the more rural localities Ahondo and Katchénou throughout the 14 months surveillance. However, among school-aged children, parasitaemia was higher in Taabo Cité than in Ahondo (Wilcoxon's rank-sum p-value: 0.008) and Katchénou (Wilcoxon's rank-sum p-value: 0.347), which suggests that children might be more susceptible to the deleterious effect of *Plasmodium* infection in areas with slightly lower infection prevalence associated to higher parasitaemia.

Although the prevalence of *Plasmodium*-hookworm co-infection among young school-aged children and young women was not significantly different from what would have been expected by chance, we found a significant positive association between both types of parasites in children and a significant negative association among young women (Righetti et al., 2012a). Advanced statistical tools (e.g. spatially explicit Bayesian modelling) might provide further precision of the relation between multiple parasitic infections in different age groups, more particularly in defining whether the observed associations are explained by environmental factors or by within-host interactions (Pullan et al., 2011; Brooker et al., 2012).

Providing an answer to the question of this section, i.e. "what is the relative contribution of parasitic infections to anaemia in Taabo HDSS" is not trivial. Overall, participants with *Plasmodia* had Hb concentrations 0.7 g/dl lower than participants without *Plasmodium* infection, which corresponds to the estimated impact that malaria control measures could

reveal on Hb concentrations in preschool-aged children (Korenromp et al., 2004). In our setting, however, infants with a *Plasmodium* infection had mean Hb concentrations 1.1 g/dl lower than their non-infected counterparts. Although we showed a significant negative correlation between Hb concentrations and the number of schistosome eggs in children, there was no significant difference in Hb concentrations in children with and without *S. haematobium* infection, at the baseline cross-sectional survey in April 2010. The increase in Hb concentrations between the baseline and the end-of-study surveys was not significantly higher in participants who have received one or several treatments against helminthiases and participants who did not receive any treatments. Moreover, data from the yearly parasitological survey done in the Taabo HDSS indicate that the overall prevalence of anaemia among individuals above 2 years was lower in June 2011 (35%) compared to June 2010 (45%). The prevalence of soil-transmitted helminth infections decreased in the entire population of Taabo HDSS between 2010 and 2011, whilst *S. haematobium* and *Plasmodium* infection prevalence were slightly higher in June 2011 compared with June 2010. The design of our study, which was not a randomised-controlled trial, does not allow us to estimate the net contribution of anthelmintic drug administration to the increased Hb concentration. Hence, our results emphasise the need of further double-blind randomised controlled trial accompanied by physical and cognitive testing and longer term monitoring, in order to elucidate the likely beneficial effect of anthelmintic drug administration on Hb concentrations (Taylor-Robinson et al., 2012). Of note, since we showed that *Plasmodium*-hookworm co-infection was associated with lower odds of cellular ID and anaemia in school-aged children, it might be worth investigating the effect of anthelmintic drug administration on Hb both in individuals with and without *Plasmodium* infection and in different age groups.

8.2.2 Diagnosis of parasitic infections in areas of low endemicity

In view of the heterogeneity and overall low intensity of helminth infections in the Taabo HDSS and other parts of Africa (Knopp et al., 2008), and current efforts towards elimination of helminthiases (Rollinson et al., 2012) more sensitive diagnostic approaches may be indicated for future research. Indeed, the overdispersion of helminth eggs in stool and daily variation in excretion require, as ideal protocol, to use replicate faecal samples over several consecutive days in order to obtain accurate estimates of helminth infection prevalence and intensity (Hall, 1981; Anderson and May, 1985; Knopp et al., 2008). Furthermore, these methods have to be specific as polyparasitism is very common in the area of Taabo and other parts of Côte d'Ivoire (Keiser et al., 2002; Raso et al., 2004, Righetti et al., 2012a). There are alternative diagnostic methods to the widely used Kato-Katz thick smear technique (WHO, 1991) which have more recently been developed or adapted to human, but they still represent considerable challenge before they can be applied in the field. Indeed, alternative

diagnostic methods are either more time consuming and more expensive than the Kato-Katz technique (e.g. FLOTAC (Cringoli et al., 2010; Speich et al., 2010)) lacking specificity (e.g. ELISA (Knopp et al., 2010)), or sensitivity (e.g. urine circulating cathodic antigen (CCA) for *S. haematobium* (Stothard et al., 2009)) or might require specialised laboratories (e.g. polymerase chain reaction (PCR)). Other diagnostic approaches, based on multiplex dipstick for the detection of trematodes, nematodes and intestinal protozoa in urine samples are still in early development stage in animal models (Li et al., 2008; Saric et al., 2008; Wang et al., 2008; Wang et al., 2009). Metabolic profiling of biological fluids samples is indeed a method which holds promise to deepen our understanding of infectious diseases through providing fingerprint and biomarkers specific to each parasitic infection. Although many years of research will most likely be necessary until these novel approaches become applicable after in-depth validation in the field, this would represent considerable advantage to diagnostic methods which require the collection of stool or blood samples.

8.2.3 Contributions of micronutrient deficiencies to the burden of anaemia in the Taabo HDSS

Micronutrient malnutrition, due to inadequate dietary intakes, repeated infections and poor bioavailability from foods, is a public health problem in many low-income countries which undermines the health, development, and economic potential of millions of people (Caulfield et al., 2006). Young children and women of reproductive age are particularly vulnerable to micronutrient deficiencies due to physiologically higher micronutrient requirements during growing and reproductive life stages, respectively (FAO 2001).

Results from our prospective longitudinal study indicate that iron, vitamin A and riboflavin deficiency are all prevalent in the Taabo HDSS, in the three age groups investigated, with the exception of vitamin A deficiency which was found in less than 10% of young women. The prevalence of micronutrient deficiency was similar at baseline and after 14 months, except among infants who showed significantly improved iron status in June 2011 compared with the baseline in April 2010. The prevalence of *P. falciparum* infection and inflammation did not change between April 2010 and June 2011 and age significantly predicted SF concentration in this age group (see Chapter 3). Hence, it is most likely that improved iron status in infants and preschool-aged children is mainly explained by growing age, inasmuch it corresponds to what has been found for similar age ranges in other sub-Saharan African countries (Stoltzfus et al., 2000; Crawley, 2004).

The prevalence of vitamin A deficiency in infants living in the area of Taabo is slightly higher to what has been estimated for other West African countries (West, 2002) and for Africa in

general (WHO, 2004). However, this observation might be explained by the younger mean age of our infant cohort compared to the total population of preschool-aged children. The prevalence of vitamin A deficiency in young women living in the Taabo area is within the range of estimates for West Africa (West, 2002). A common clinical outcome of vitamin A deficiency is night blindness and, for more severe cases, xerophthalmia. Furthermore, vitamin A deficiency is a risk factor for increased severity of infectious disease and mortality (Caulfield et al., 2006). In our study, we did not record xerophthalmia cases and hence cannot estimate the prevalence of clinical vitamin A deficiency. However, our results confirm a significant association between vitamin A deficiency and malaria (SanJoaquin and Molyneux, 2009), emphasising the importance of sustained effort to control this micronutrient deficiency. In Côte d'Ivoire, the polio immunisation programme administers supplements of vitamin A concomitantly to the three doses of polio vaccine to infants before their first birthday. However, the high prevalence of vitamin A deficiency in the study area suggests that, within the socio-political context of Côte d'Ivoire, these measures are not sufficient to control vitamin A deficiency in preschool-aged children.

Riboflavin deficiency is mainly found among population consuming little milk or meat products but there are few data for West Africa (Reddy et al., 1987; Lartey et al., 2000; Wegmüller et al., 2006). Our data are consistent with findings from nearby villages (Rohner et al., 2007) and indicate that the diet in Taabo is qualitatively and/or quantitatively not adequate to fulfill micronutrients requirement and recommended dietary allowance (RDA) considering the high endemicity of infectious and inflammatory diseases.

Attempting to answer the question of this section, i.e. “what is the relative contribution of micronutrient deficiency to anaemia in the Taabo HDSS”, the following issues are offered for consideration. Pooling all data from participants without inflammation in our longitudinal monitoring, Hb concentrations were, on average, 0.7 g/dl lower in individuals with cellular ID, 1.4 g/dl lower in participants with storage iron depletion and 1.6 g/dl in participants suffering from both cellular ID and storage iron depletion. These observations emphasise the already acknowledged contribution of ID to anaemia in population groups highly at risk.

8.2.4 Estimation of micronutrient status in areas with high prevalence of inflammation and *Plasmodium* infection

In our setting, adjusting SF and sTfR concentrations for specific inflammatory stages (incubation, early and late convalescence) significantly modifies the estimated prevalence of ID in infants and school-aged children. Adjusting sTfR for *P. falciparum* infection significantly increased the estimated prevalence of ID in children at early school-age.

We re-assessed the association between demographic, socioeconomic, nutritional and parasitic variables and anaemia for the baseline survey in April 2010, to see whether integrating correction factors might modify initial significant associations. We found the exact same results: *P. falciparum* infection was significantly associated with anaemia in infants; young school-aged children with cellular iron deficiency and chronic inflammation had significantly higher odds of anaemia and cellular ID, severe riboflavin deficiency and working at home were significantly associated with anaemia in young women. These observations indicate that the effect of inflammation and *P. falciparum* on iron biomarkers do not alter our findings and suggest that our results are robust and plausible and might be communicated at higher and broader levels (e.g. Ivorian Ministry of Health, *Programme National de Lutte contre le Paludisme*, health staff working in the Taabo HDSS).

A Cochrane review indicated that inflammation might decrease retinol concentrations as many as 24% (Thurnham et al., 2003). Future studies should integrate correction factors and adjust retinol concentrations for inflammation in order to assess more accurately the relation of vitamin A deficiency and anaemia or *Plasmodium* infection.

Given the effect of inflammation on various micronutrient biomarkers, there is an urgent need of reviewing the guidelines which define how micronutrient status should be estimated at the population level. Since inflammation and malnutrition often coexist in sub-Saharan Africa, it is likely that adjusting sTfR, SF and serum retinol values for inflammation will modify the current estimated prevalence and relative attributed disease burden.

8.2.5 Contributions of other factors to the local burden of anaemia

Our prospective longitudinal study focussed on several micronutrient deficiencies and parasitic infections. However, other micronutrient deficiencies as well as other pathogens might exert an important contribution to the local burden of anaemia in south-central Côte d'Ivoire and elsewhere in the developing world.

Infants, school-aged children at early school age and young women with inflammation (defined as AGP >1g/l or CRP >5 mg/l) showed significantly higher odds of anaemia, either at baseline or at the end-of-study survey. Individuals with inflammation had, on average, 0.7 g/dl lower Hb concentrations. In individuals free of *Plasmodium*, this difference was lower (0.4 g/dl). Although *P. falciparum* parasitaemia significantly positively correlate with AGP and CRP in the infant- and school-aged child-cohorts, 40%, 14% and 43% of infants, school-aged children and women with inflammation, respectively, did not harbour any *Plasmodium*, as defined by a positive RDT or the presence of *Plasmodium* on a Giemsa-stained blood film examined under a microscope. This observation emphasises that other pathogenic agents

contribute to the burden of subclinical inflammation in our study area. Moreover, there is a growing body of evidence that suggests that, at present, a considerable number of fevers in African individuals are not caused by malaria (D'Acremont et al., 2010; Gething et al., 2010). Bacteria and viruses (e.g. agents of acute respiratory infections, measles, HIV/AIDS, gastroenteritis, and urinary tract infections) might also produce subclinical or clinical inflammation, particularly in non-immune children. Further research and in-depth investigations are needed to determine which specific pathogenic agents that trigger inflammatory reactions are endemic in the Taabo HDSS and elsewhere in sub-Saharan Africa. Pregnant women showed mean Hb concentrations 1g/dl lower than non-pregnant women, confirming the higher risk of anaemia related to pregnancy.

We measured vitamin B12 and folate concentrations in a sub-sample of 153 fasted individuals in June 2011 and found a prevalence of folate deficiency of 0%, 3% and 32% in infants, school-aged children and women. We found no deficiency in vitamin B12 among infants and women and the prevalence in school-aged children was 2%. Considering the small sample size, integrating these variables in statistical analyses might produce biased results. Nevertheless, the high prevalence of folate deficiency in young women suggests that this parameter might be important to consider in future studies. Indeed, as folate represent a very important micronutrient during pregnancy, it is important to assess folate status among women of childbearing age.

8.3 Control and intervention programmes targeting anaemia

The overall goal of this PhD thesis was to further our understanding of the aetiology of anaemia among three age groups in the Taabo HDSS. Our results do not provide direct evidence on new interventions to be implemented in order to decrease the prevalence of anaemia in Côte d'Ivoire. Yet, there are several specific interventions (e.g. LLINs, IRS, prompt diagnosis of malaria and effective treatment using artemisinin-based combination therapy, nutritional education, improved iron intake and food fortification) which have already shown potential in decreasing the prevalence of anaemia in similar settings (Lartey 2008, Alaofè et al., 2009; Terlouw et al., 2010; De-Regil et al., 2011; Suchdev et al., 2012). Other interventions such as community-led total sanitation are complementary tools which have the potential in exerting broad impact on population's health and wellbeing. Some of these interventions are currently being implemented within the Taabo HDSS and it will be interesting to closely monitor the effect of these interventions on people's health.

The results of our survey about cultural concepts of various anaemia-related illnesses and their relationship with people's behaviour (Kouadio et al., 2013) might be useful for more effective and efficient development and implementations of interventions targeting the major aetiological agents of anaemia (e.g. *Plasmodium* infection, ID and inflammation). Indeed, in order to be efficient, these interventions should consider the taxonomy of local diseases and use appropriate local terminology (Winch et al., 1996; Granado et al., 2011). Moreover, a considerable effort should be made in order to facilitate and ameliorate the communication between the health system and the population. Targeted awareness and education campaigns have recently been conducted in several localities of Taabo HDSS, focussing on sanitation and hygiene. Further health education tools, with particular emphasis on malaria and nutrition, might exert additional beneficial effect on the people's health (Walsh et al., 2002). Considering the hierarchical and, to some extent, patriarchal functioning of the communities living in the Taabo area, the "administration" of education interventions should not be restricted to health dispensary or schools but also involve communities and village chiefs. Finally, the sustainability of health interventions will depend on the implication of the community during the planning and the implementation of the interventions.

8.4 Public health implications

Anaemia is a worldwide public health problem, disproportionately affecting young infants and women of childbearing age in low- and middle-income countries. Although anaemia is multifactorial, the local aetiology depends on specific environmental (including genetic background), sociologic and economic parameters. We note, however, that there are several variables that affect the risk of anaemia at a global level (**Figure 8.2**).

At a global scale, socioeconomic issues, the natural environment (e.g. altitude), population movements, urbanisation, food systems, family planning (e.g. pregnancies spacing), age and gender are parameters which might affect, directly or indirectly, Hb concentrations. The agricultural and health systems, food habits, *Plasmodium* and soil-transmitted helminth endemicity and haemoglobinopathies are regional parameters which govern the risk of anaemia (Verhoef et al., 2002; Crawley, 2004; Tolentino and Friedman, 2007; Ouedraogo et al., 2011; Sousa-Figueiredo et al., 2012). Local or individual factors affecting Hb concentrations include the socioeconomic status, focal parasitic diseases (e.g. schistosomiasis), micronutrient status (e.g., iron, folate, vitamin B12, vitamin A and riboflavin status) and potentially gene-environment interactions (Dominguez-Salas et al., 2012).

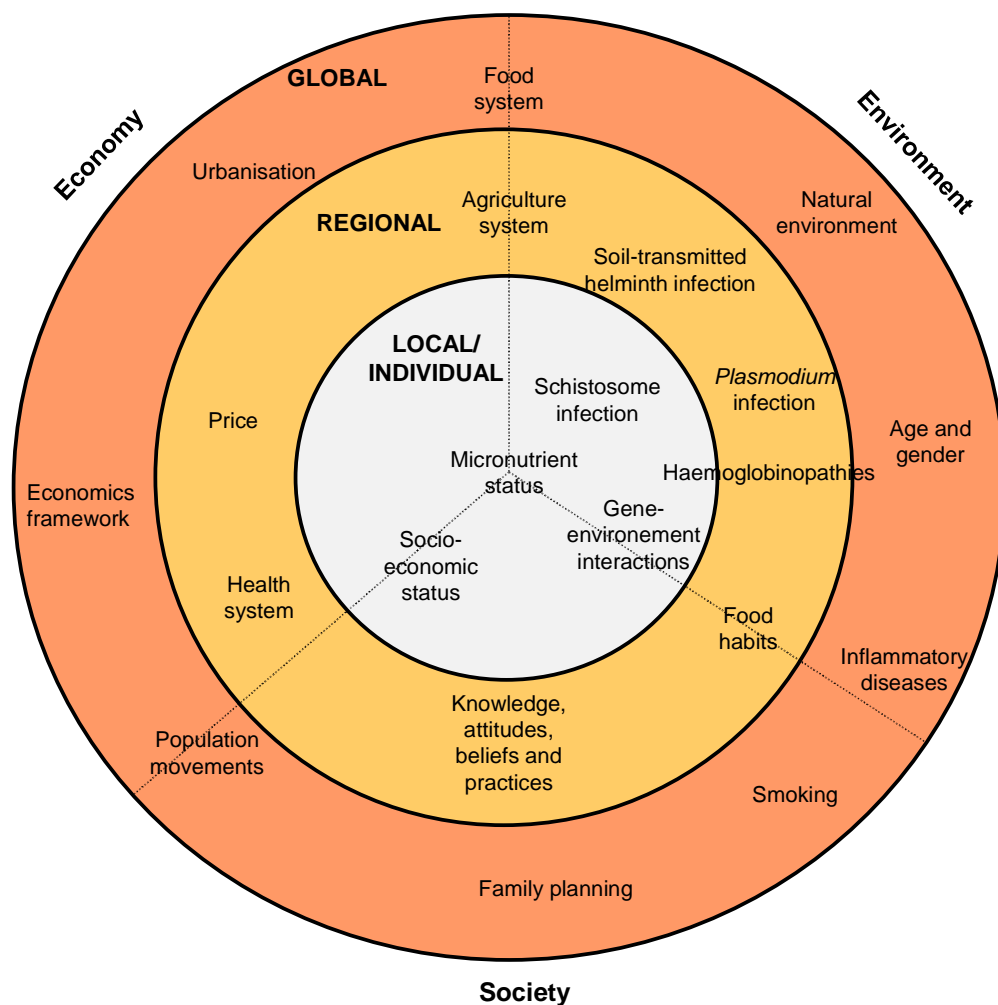


Figure 8.2: Global framework of economic, environmental and sociologic parameters which might affect Hb concentrations at a global, regional and local scale.

Recently, a couple of studies have investigated the sub-regional variance in Hb concentrations and the relative contribution of several risk factors for anaemia in preschool-aged children throughout sub-Saharan Africa (Soares Magalhães and Clements 2011a, 2011b). The results indicate that the major risk factors are very similar throughout West Africa, but that there is a considerable variance in Hb mean concentrations within African subregions. Importantly, the geographically mapped prevalence of severe anaemia (Hb <7g/dl) shows a relative heterogeneity across West Africa. I will argue here that these results are of practical relevance for targeted cost-effective control efforts.

Our data stem from a specific social-ecological system in south-central Côte d'Ivoire, where tropical climate meets Savannah. Attempting to extrapolate our results to other areas of Côte d'Ivoire, West Africa or elsewhere in the developing world would make little sense due to setting-specific variations. Yet, several health issues might be important at a regional scale.

Indeed, in malaria endemic areas, a considerable fraction of severe anaemia cases is attributable to malaria (Guyatt and Snow, 2001; Murphy and Breman, 2001). Moreover, ID and inflammatory diseases from parasites, bacteria and viruses are endemic across West Africa. The local concepts we identified about anaemia might also be considered at a regional scale since we showed that all ethnic groups of Côte d'Ivoire as well as migrants from other West African nations utilize the concepts of "blood is finished" to describe anaemia.

Moreover, several studies have shown that several, rather than one single term relate to malaria across sub-Saharan Africa (Winch et al., 1996; Ahorlu et al., 2005). The ambiguity we identified between the health staff and the population about nutritional issues is obviously not restricted to the area of Taabo. Hence, the findings that malaria and ID are important contributors to the burden of anaemia in school-aged children and young women might be extrapolated to West Africa more broadly. The severity and relative contributions of these factors and other focal diseases (e.g. schistosomiasis and HIV/AIDS) might show sub-regional idiosyncrasies and potential interactions might be specific to the location or even to the level of an individual. Bayesian geostatistical modelling might be useful for fine-tuning specific national control programme strategies. Such statistical models should not only investigate the effect of environmental parameters (e.g. elevation, land surface temperature, normalised difference vegetation index, distance to a perennial water body, etc.) on Hb concentrations but adjust them for the socioeconomic status (including education) of rural and urban populations.

8.5 Conclusions

The overarching goal of this PhD thesis was to deepen the understanding of the aetiology of anaemia in infants, school-aged children and young women in a primarily rural setting of south-central Côte d'Ivoire. Our 14-month prospective longitudinal monitoring placed particular emphasis on the dynamics of anaemia in relation to socio-demographic, parasitic and nutritional parameters. These biomedical and demographic investigations were complemented with a more qualitative piece, studying local concepts related to anaemia and assessing their relation to school-aged children and women's behaviours.

Research covered in this thesis provides detailed characterization of the three main settings of the Taabo health demographic surveillance system (i.e. small town, village and hamlet) in terms of *Plasmodium* and helminth infections and nutritional and inflammatory status of the three selected population cohorts. As per the design of our study, we utilized anaemia as outcome and determined how demographic, parasitic and micronutrient status parameters govern the dynamics of this outcome with specific measures taken at a baseline and during a 14-month follow-up period with intermediary appraisal once every 3-4 months. Importantly, during the study, there were community-based interventions, most importantly preventive chemotherapy with albendazole and ivermectin targeting soil-transmitted helminthiases and lymphatic filariasis (June 2010) and individual treatments to treat clinical malaria and schistosomiasis. The data from our prospective longitudinal monitoring allowed us to assess the effect of adjusting sTfR and SF concentration for inflammation and *P. falciparum* infection on the estimated prevalence of ID. The KAPB study was conducted to investigate local concepts of anaemia, their relation with people's behaviour and to discuss the potential implications these relations have for public health. Based on the results from the work conducted for this thesis, a set of conclusions are offered for consideration:

- Infants, school-aged children and young women in the Taabo HDSS suffer from high prevalences of anaemia, *P. falciparum* infection, inflammation and deficiencies of iron, riboflavin and vitamin A, but low prevalences and intensities of soil-transmitted helminth and schistosome infections.
- Considering the results of the baseline and the end-of-study surveys, there is a significant recurrent association between anaemia and cellular ID and inflammation in the three age groups studied. Moreover, *P. falciparum* is significantly and highly associated with anaemia in infants below the age of 2 years. These results call for effective prevention and control measures targeting *P. falciparum* in young infants

and, more generally, ID. These interventions might include the distribution of LLINs, a better access to prompt diagnosis and quality treatment against malaria, improved intake of bioavailable iron, and anthelmintic drug administration coupled to health and nutritional education.

- The prevalence of *P. falciparum* infection and parasitaemia was relatively stable throughout the 14-month monitoring period whilst helminth infection prevalence significantly decreased during the study, most likely due to preventive chemotherapy. These results indicate that control measures targeting *P. falciparum* and helminth infections at the population level should be implemented or sustained.
- Hb concentration was significantly higher in June 2011 compared with April 2010 in infants and school-aged children, and a similar trend was observed in women. Whilst the constant increase in Hb concentration observed in infants throughout the 14 months most likely reflects a gradual improvement in iron status as the iron supply was better able to meet needs once growth had slowed down, the dynamics of anaemia in school-aged children and women was more complex and affected by external factors.
- Adjusting sTfR concentration for inflammation significantly decreased the estimated prevalence of ID in infants and school-aged children. Adjusting sTfR concentration for *Plasmodium* infection decreased ID prevalence in school-aged children. Indeed, sTfR and SF provide very different estimates of ID prevalence, and hence caution is currently indicated if these biomarkers are being used to estimate the prevalence of ID and IDA in areas with high prevalence of subclinical inflammation or *Plasmodium* infection.
- We identified local terms which referred to the concept of anaemia in all communities, and the population acknowledged anaemia as an important public health problem. The overall concept, knowledge and behaviours related to anaemia were similar across study settings and between participants who have been exposed to prior research and newly recruited participants. Although an estimated 44% of the people of Taabo HDSS is sleeping under LLINs, *djékouadjo* was not acknowledged as disease exclusively transmitted by mosquitoes. Most participants did not have a varied diet the day preceding the interview and people generally referred to the quantity, rather than the quality, of food when talking about nutritional issues. These results call for a better communication between the health staff and the population to improve the acceptance and effectiveness of future health interventions.

8.6 Research needs and recommendations

8.6.1 Identified research needs

The work of the present PhD thesis contribute to a better understanding of anaemia, inasmuch as it allowed to (i) identify specific associations between anaemia and parasitic, nutritional and socio-demographic parameters; (ii) show that multiple parasitic infections do not necessarily worsen haematological and iron status parameters in comparison with a single infection; (iii) describe the dynamics of Hb in relation to age, parasitic infections and micronutrients status over a 14-month period; (iv) highlight that inflammation affects sTfR concentrations, independently of *Plasmodium* infection; and (v) identify local concepts related to anaemia, including the assessment of how these might affect people's behaviours and, in turn, have implications for public health in Côte d'Ivoire. Issues emerging from our study for future research, including basic and applied research, are detailed below.

- Identify which infections (other than *Plasmodium*) are associated with subclinical inflammation in infants and school-aged children. Future studies should pay greater attention to differential diagnosis of intestinal protozoan, bacterial and viral diseases.
- Reassess the prevalence of anaemia in our study participants, one or two year after the end-of-study survey and compare it with the prevalence of anaemia in matched individuals in the Taabo HDSS to determine the potential long-term beneficial effect of health interventions targeting malaria and helminthiases.
- Investigate the exact relative contribution of parasitic infections (e.g. *Plasmodium* and helminths) and micronutrient deficiencies to anaemia through an intervention study with a factorial design in infants and school-aged children. Since previous studies with similar interventions did not show any important significant effect on Hb concentrations, effective iron compounds might be coupled to other micronutrients and ACTs, instead of SP, should be used.
- Analyse urine and plasma samples collected during our prospective longitudinal study through proton nuclear magnetic resonance (¹H-NMR) or mass spectrometry (MS) to validate markers of *Plasmodium* infection identified in mice and identify further specific and sensitive biomarkers for other infections. The ultimate goal is to develop multiplex dipsticks allowing to diagnose several infections in a single urine sample.

- Monitor the implementation, on the local market, of new iron fortified condiment cubes and assess the effect of their distribution on populations' health (Nestlé 2012).
- Investigate if women and their newborns who have close and frequent contact with fire have lower Hb concentration. Assess the effect of indoor and outdoor biofuel smoke on Hb level of women and young infants. Quantify the level of exposure of mother and children to harmful particles (e.g. CO and small particular matter < 2.5 µm).
- Several plants from the families Malvaceae, Solanaceae, Acanthaceae, Lamiaceae and Poaceae are locally used in the prevention and treatment of anaemia. Pharmacognosy studies might investigate potential active components in these plants.
- Compare the sensitivity, specificity, positive and negative predictive values of sTfR for predicting iron stores in infants compared with stained iron as 'gold' standard.
- Measure SF and sTfR during the course of an inflammatory reaction and define when it comes back to normal values (exact dynamics). This experiment may require a first pilot study in animal models with many time-points before human studies.
- Assess the intake of bioavailable iron through food records in households from different settings and socioeconomic status across Taabo HDSS.
- Compare the genome-wide expression in RBC or plasma from mice before and after iron-restricted diet to detect potential new biomarkers of nutritional ID
- Investigate the epigenetic effect of malnutrition or specific vitamin deficiency during pregnancy on the health of the child and during childhood on later health status.

8.6.2 Recommendations

In this latest section, I introduce some suggestions on how to better prevent and control anaemia in the Taabo HDSS. Although an integrated, non disease-specific approach would be optimal, I specify the likely term to interventions under brackets.

- Health system strengthening with implication of the community (ongoing)
- Community-led total sanitation (pilot study ongoing).
- Regular administration of free anthelmintic treatments at the population levels (ongoing).
- Evaluation of the number of LLINs at the national level, distribution in areas where coverage is insufficient, accompanied by basic explanations about malaria (short).
- Increasing use of antiretroviral treatment (short).
- Construction of community pumps (medium).
- Implementation of RDTs and ACTs for enhanced malaria control in rural health dispensaries (medium), facilitate antenatal cares and IPT during pregnancy.
- Sustain and reinforce national programmes of vitamin A supplementation during early childhood; extend to pregnancy (medium to long).
- Health education (medium to long), placing particular emphasis on parasitic diseases (within school and communities).
- Nutritional education (medium to long), with an emphasis on diet diversification and food preparation techniques.
- Development of a community nutrition programme (medium to long).
- Integration of affordable and effective iron fortified food or multivitamin powders in the local “boutiques” (long).

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9. Curriculum vitae

AURELIE ASSUNTA RIGHETTI

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 Swiss • 1984, July 29th

EDUCATION

- 7/2009 – 9/2012 **PhD in Epidemiology** – Swiss Tropical and Public Health Institute (Swiss TPH), University of Basel, Basel, Switzerland.
 PhD thesis Aetiology of anaemia and public health implications in the Taabo health demographic surveillance system, south-central Côte d'Ivoire (Supervision: Prof. Dr. Jürg Utzinger, SwissTPH/University of Basel; Dr. Rita Wegmüller and Prof. Dr. Richard F. Hurrell, ETHZ; Prof. Dr. Eliézer K. N'Goran, Université Félix Houphouët-Boigny/ Centre Suisse de Recherches Scientifiques en Côte d'Ivoire).
- 9/2007 – 2/2009 **MSc in Genomics and Experimental Biology** – Centre for Integrative Genomics, University of Lausanne, Lausanne, Switzerland.
 MSc thesis Hypoxia-induced angiogenesis: understanding the interaction between HIF-1 alpha and PPAR beta (Supervision: Dr. Liliane Michalik, Center for Integrative Genomics, University of Lausanne).
- 9/2004 – 7/2007 **BSc in Biology** – University of Lausanne, Lausanne, Switzerland.
- 8/2000 – 7/2003 **High School** (bilingual French-German) – High School Auguste Piccard, Lausanne, Switzerland.
 Emphasis Spanish, Biology

PROFESSIONAL EXPERIENCE

- 7/2009 – 12/2012 **Research Fellow/ PhD Candidate** – Swiss TPH, Basel, Switzerland ; ETH Zurich, Zurich, Switzerland ; Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, Côte d'Ivoire.
Designed and implemented a 14-month prospective longitudinal monitoring to investigate the aetiology of anaemia in the Taabo health demographic surveillance system, south-central Côte d'Ivoire. Managed a team of 15 people, logistics and public relations. Led laboratory and statistical analysis. Published first-author manuscripts in international peer-reviewed journals. Presented results at meetings.
- 5/2009 – 6/2009 **Intern** – Institute for Preventive Medicine and Institute of Plants Physiology and Genetics, Dushanbe, Tajikistan.
Helped for serological laboratory diagnosis and for the collection of field specimen.
- 3/2009 – 4/2009 **Teacher** at the secondary school of Grandson (French, History, Natural Sciences), Grandson, Switzerland.

7/2007 – 8/2007 **Consultant** – Regio21, local network for sustainable development
Developed and implemented an informative web resource in a sustainable development perspective (www.regio21.ch part “Ecoles”).

2006 – 2007 **Student Assistant** – Department of Ecology and Evolution, Prof. Dr. Laurent Keller, University of Lausanne.

COMMUNITY SERVICE AND MEMBERSHIPS

7/2003 – 9/2004 Travels and (voluntary) work in Burkina Faso, Cuba, Dominican Republic, Ghana, India, Mali and Switzerland.

1/2002 – 7/2002 Fundraising in Switzerland with the foundation *Nouvelle Planète* and construction of a classroom in the *Centre Apicole Selintaanba* in Fada N’Gourma, Burkina Faso.

Member of the Swiss Society of Parasitology and Tropical Medicine

Member of “Association Suisse-Mali” (www.suisse-mali.ch)

Member of ArnonBike (www.arnonbike.ch)

LANGUAGES AND OTHER SKILLS

Languages French (native); German and English (fluent); Spanish (intermediate)

Other skills MS Office, STATA, Mouse dissection, Cell culture, (RT) PCR, Western Blot, ELISA, IMMULITE, EGRAC, HPLC, Certificate of Good Clinical Practices

PEER-REVIEW AND PRESENTATION ACTIVITIES

External reviewer for *Parasites and Vectors* and the *Journal of Infectious Diseases* (2012)

Presentation at the student seminar of the Department of Epidemiology and Public Health of the Swiss TPH about “Aetiology of anaemia in infants, school-aged children and young women in south-central Côte d’Ivoire” (08/2012)

Organisation of a restitution and information meeting with the communities’ chiefs in Taabo Cité, Ahondo and Katchénou. Presentation entitled “Rechercher les causes de l’anémie dans le SSDS de Taabo” (01/2012)

Co-organisation and talk on “Aetiology of anaemia and potentials of metabonomics” at the workshop “Exploring capacity for metabolic profiling in Côte d’Ivoire” at CSRS, in Abidjan (01/2012)

Talk at the 2011 meeting of the Swiss Society of Parasitology and Tropical Medicine about “Aetiology of anaemia among infants, school-aged children, and young non-pregnant women in different settings of Côte d’Ivoire” (11/2011)

Organisation of a restitution and information meeting with the communities’ chiefs in Taabo Cité. Presentation entitled “Rechercher les causes de l’anémie dans le SSDS de Taabo” (10/2010)

PUBLICATIONS

Righetti AA, Wegmüller R, Glinz D, Ouattara M, Adiossan LG, N’Goran EK, Utzinger J, Hurrell R (2013). Effects of inflammation and *Plasmodium falciparum* infection on soluble transferrin receptor and plasma ferritin concentration in different age groups: a prospective longitudinal study in Côte d’Ivoire. *American Journal of Clinical Nutrition* 97: 1364-1374

Kouadio MKD, **Righetti AA**, Abé NN, Wegmüller R, Weiss MG, N’Goran EK, Utzinger J (2013). Local concepts of anemia-related illnesses and their public health implications in the Taabo health demographic surveillance system, south-central Côte d’Ivoire. *BMC Hematology* 13:5

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Righetti AA, Glinz D, Adiossan. GL, Koua AGK, Niamké S, Hurrell RF, Wegmüller R, N’Goran EK, Utzinger J (2012). Interactions and potential implications of *Plasmodium*-hookworm coinfection in different age groups in south-central Côte d’Ivoire. *PLoS Neglected Tropical Diseases* 6(11): e1889.

Righetti AA, Koua AG, Adiossan, GL Glinz D, Niamké S, Hurrell RF, Wegmüller R, N’Goran EK, Utzinger J (2012). Etiology of anemia among infants, school-aged children and young non pregnant women in south-central Côte d’Ivoire. *American Journal of Tropical Medicine and Hygiene* 87(3):425-434

Müller I, Coulibaly JT, Fürst T, Knopp S, Hattendorf J, Krauth SJ, Stete K, **Righetti AA**, Glinz D, Yao AK, Pühse U, N’Goran EK, Utzinger J (2011). Effect of helminth infections and malaria on physical fitness of school children in Côte d’Ivoire. *PLoS Neglected Tropical Diseases* 5(7): e1239.

10. Appendix

10.1 Questionnaire administrated to children who participated in the KAPB study

ETIOLOGIE DE L'ANEMIE DANS LE DSS DE TAABO- ENQUETE SOCIOLOGIQUE- JANVIER/FEVRIER 2012

QUESTIONNAIRE SOUMIS AUX *ENFANTS*

DATE DE L'ENTRETIEN : ___/___/_____	LOCALITE : _____
NOM DE L'ENQUETEUR. _____	
NOM DU PARTICIPANT. _____	
ID DSS _____	ID ANEMIE. _____
Consentement écrit (empreinte digitale de l'enfant et d'un parent): /_____/ /_____/	

I- PROFIL SOCIO-DEMOGRAPHIQUE			
1. Langue de l'entretien:	_____		
2. Scolarisation	/___/ (01=scolarisé, 02=non scolarisé, 09=ne sait pas)		
3. Niveau d'alphabétisation:	/___/ (01=ne sait ni lire ni écrire, 02=sait lire, 03= sait lire et écrire)		
4. Religion	/___/ (01=chrétienne, 02=musulmane, 03=animiste, 04=autre)		
II- CONNAISSANCE DE L'ANEMIE (« MANQUE DE SANG »)			
5. C'est quoi le sang ? _____			
6. Tu connais l'« anémie »? (01=oui; 02= non; 09= nsp) /___/ si l'enfant répond « oui », demande-lui « c'est quoi ? » _____			
7. Tu as déjà entendu parler de « manque de sang » ? (01=oui; 02= non; 09= nsp) /___/			
8. Si oui, comment on dit « manque de sang » dans ta langue ? _____			
9. Tu as déjà entendu parler de «manque de sang» (utiliser le terme en baoulé ou dioula)? : (01=oui; 02= non; 09= nsp) /___/			
<i>si le participant répond NON aux questions 6,8 ET 9, le questionnaire est terminé</i>			
10. Où/comment as-tu entendu parler de ça ? _____			
<i>Réponses dirigées : (01=oui, 02=non, 09=nsp)</i>			
a. centre de santé /___/	b. école /___/	c. campagne de sensibilisation /___/	d. famille /___/
f. ami(e)/___/	g. télévision /___/	h. radio /___/	
i. étude anémie /___/	j. autre(à préciser) : _____		

11. Qu'est ce qu'on t'a dit sur le manque de sang ?

Réponses dirigées : (01=oui, 02=non, 09=nsp)

- a. maladie grave /__/ b. c'est bon /__/ c. maladie mystique /__/ d. maladie pas grave /__/

12. Pour toi, c'est quoi le « manque de sang » ? ça fait quoi ?

Réponses dirigées : (01=oui, 02=non, 09=nsp)

- a. la mort /__/ b. la sorcellerie /__/ c. la maladie /__/ d. la santé /__/ e. le bonheur /__/

II-1- ETIOLOGIE

13. Qu'est ce qui donne l'anémie?

Réponses dirigées (01= oui ; 02=non ; 09=ne sait pas)

- a. Le soleil /__/ b. le paludisme (« djékouadjo ») /__/ c. les sorciers /__/ d. la grossesse /__/
 e. une alimentation qui n'est pas bonne /__/ f. des saignements abondants (ex : blessures) /__/ g. le feu /__/
 h. Le non respect d'interdits /__/ i. Manque d'hygiène /__/ j. des vers dans le ventre /__/ k. ictère /__/
 k. La bilharziose (pisse du sang) /__/ l. le SIDA /__/ m. des maladies héréditaires (l'enfant naît avec) /__/

II-2- SYMPTÔMES DE L'ANEMIE

14. Comment tu reconnais quelqu'un qui manque de sang ?

Réponses dirigées : (01=oui, 02=non, 09=nsp)

- a. couleur de l'intérieur de l'œil (la personne qui administre le questionnaire montre sur soi-même) /__/
 b. couleurs des lignes dans la paume de la main (la personne qui administre le questionnaire montre sur soi-même) /__/
 c. couleurs de l'ongle (la personne qui administre le questionnaire montre sur soi-même) /__/
 d. pâleur du corps /__/ e. fatigue, somnolence /__/ f. faiblesse /__/ g. vertiges /__/
 h. cœur qui bat vite /__/ i. perte de poids /__/ j. il est gros /__/ k. il court vite /__/

II-3- CONSEQUENCES DE L'ANEMIE

15. Le manque de sang, ça donne quoi?

Réponses dirigées : (01=oui, 02=non, 09=nsp)

- a. fatigue, faiblesse /__/ b. difficultés à l'école (concentration, apprentissage...) /__/ c. la force /__/
 d. problèmes durant la grossesse ou à l'accouchement /__/
 e. maladies /__/ f. la mort /__/ g. la santé /__/

II-4- PERSONNES LES PLUS VULNERABLES

16. C'est quel groupe le manque de sang fatigue plus?

Réponses dirigées : (01=oui, 02=non, 09=nsp)

- a. enfants de moins de 5 ans /__/
 b. enfants qui vont à l'école /__/ c. jeunes hommes /__/
 d. jeunes femmes /__/ g. femmes enceintes /__/ f. vieillards /__/

III- EXPOSITION A L'ANEMIE

17. Tu as déjà eu ça, le manque de sang ? (01=oui, 02=non; 09=nsp) /__/

Si la personne répond « NON », passez à la question 22

18. Si oui, combien de fois? /___/ fois

19. Comment tu as su? Qui t'a dit que tu avais ça ? /__/(01= propre jugement, 02=visite médicale, 03=tradipraticien, 04=information par un tiers, 05. autre, à préciser : _____)

20. Ca t'a fait quoi ?.....

Réponses dirigées : (01=oui, 02=non, 09=nsp)

- a. fatigue /__/ b. force /__/ c. maladies /__/, précisez : _____
 d. difficultés à l'école/aux champs /__/ e. arrêt de travail/école /__/

21. Ca a mis combien de temps ? /___/ jours OU /___/ mois OU /___/ années

IV- PREVENTION DE L'ANEMIE

22. C'est quoi on peut faire si on ne veut pas avoir manque de sang?

Réponses dirigées : (01=oui, 02=non, 09=nsp)

- a. Consultations prénatales /__/ b. Médecine traditionnelle /__/ c. Alimentation variée /__/
 d. Allaitement exclusif durant les 6 premiers mois de vie de l'enfant /__/ e. Prières, pratiques spirituelles /__/
 f. Se protéger du paludisme /__/ g. Objets de santé /__/ h. Faire un contrôle annuel à l'hôpital /__/
 i. manger des tomates /__/ j. boire des sucreries /__/

V- ITINERAIRE THERAPEUTIQUE

Si la personne a répondu « NON » ou « ne sait pas » à la question 17, passez à la question 28

23. Quand tu as eu ça, on a fait quoi pour te soigner ? _____

24. On t'a donné médicament (médecine traditionnelle ou moderne)? (01=oui, 02=non, 09=nsp) /__/

25. Tu as reçu ou tes médicaments ?

- a. Hôpital, centre de santé /__/ b. famille /__/ c. Pharmacie (dépôt) /__/ d. médicaments de la rue /__/.
 f. Tradipraticien /__/ g. autre /__/

26. Si possible, précisez le nom du traitement reçu: _____

27. Qu'est ce qui a été plus efface pour toi ? _____

VI- ABITUDES ALIMENTAIRES

28. Hier tu as mangé quoi (du matin jusqu'au soir)?

Matin : _____

Midi : _____

Soir : _____

Autres : _____

29. Est-ce qu'il y a des repas ou des boissons qu'on peut prendre pour ne pas avoir l'anémie ? (01=oui, 02=non, 09=nsp) /__/

30. Si oui, lesquels ? _____

31. Les aliments/les boissons suivant(e)s sont-ils importants dans la prévention de l'anémie ? (01=oui, 02=non, 09=nsp)

a. Viande, rognons./__/ b. Poisson./__/ c. oeuf. /__/ d. sucreries/__/ e. attiéké /__/

f. fruits (mangues, ananas, papayes, oranges, banane) /__/ g. kabato /__/ h. bissap /__/

i. tomates (tomates)/__/ j. oignons, aubergines, choux /__/ k. le vin /__/

VII- INTERDITS ALIMENTAIRES

32. Est-ce qu'il y a des repas/viande que tu ne DOIS pas manger? (01=oui, 02=non, 09=nsp) /__/

33. Si oui, lesquels ? _____

34. Pourquoi on t'a dit de ne pas manger ça? (01=oui, 02=non, 09=nsp)

a. éviter des maladies /__/ b. interdit familial ou communautaire /__/ c. autres, à préciser : _____

😊😊😊😊😊😊 MERCI BEAUCOUP POUR TA PARTICIPATION !!! 😊😊😊😊😊😊

AS-TU DES QUESTIONS?

10.2 Questionnaire administrated to women who participated in the KAPB study

ETIOLOGIE DE L'ANEMIE DANS LE DSS DE TAABO- ENQUETE SOCIOLOGIQUE- JANVIER/FEVRIER 2012
QUESTIONNAIRE SOUMIS AUX JEUNES FEMMES

DATE DE L'ENTRETIEN : ____/____/____ LOCALITE : _____
 NOM DE L'ENQUETEUR : _____
 NOM DU PARTICIPANT : _____
 ID DSS ____ - ____ - ____ - ____ - ____ ID ANEMIE. ____ - ____ - ____
 Consentement écrit (empreinte digitale) : _____

I- PROFIL SOCIO-DEMOGRAPHIQUE

1. Langue de l'entretien: _____
2. Statut matrimonial /_/ (01=mariée, 02=célibataire, 03=veuve)
3. Niveau d'instruction /_/ (01=sans instruction, 02=primaire, 03=secondaire, 04=supérieur, 05= autre)
4. Niveau d'alphabétisation : /_/ (01=ne sait ni lire ni écrire, 02=sait lire, 03= sait lire et écrire)
5. Profession /_/ (01=agricultrice, 02=commerçante, 03=ménagère, 04=élève, 05=autre, à préciser : _____)
6. Religion /_/ (01=chrétienne, 02=musulmane, 03=animiste, 04=autre)
7. Nombre de grossesse(s) : /_/ /_/
8. Nombre d'enfant(s) vivant(s): /_/ /_/
9. Nbre de personne(s) dans le ménage : /_/ /_/
10. Facilités du ménage (01= oui ; 02=non ; 09=ne sait pas)
 Electricité /_/ Toilettes /_/ Puits /_/ Radio /_/ Télévision /_/ Lecteur vidéo /_/
 Ventilateur /_/ Climatisation /_/ Réfrigérateur /_/ Vélo /_/ Moto /_/ Voiture /_/

II- CONNAISSANCE DE L'ANEMIE (« MANQUE DE SANG »)

11. Que représente le sang pour vous ?

12. Est-ce que vous avez déjà entendu parler d'« anémie » ? (01=oui; 02= non; 09= nsp) /_/
- SI NON ; PASSER A LA QUESTION 14**
13. Si oui, comment dit-on « anémie » dans votre langue ? _____
14. Avez-vous déjà entendu parler de « manque de sang » ? (01=oui; 02= non; 09= nsp) /_/
15. Si oui, comment dit-on « manque de sang » dans votre langue ? _____
16. Avez-vous déjà entendu parler de «manque de sang» (utiliser le terme baoulé ou dioula)? : (01=oui; 02= non; 09= nsp) /_/
- si le participant répond NON aux questions 12, 14 et 16, le questionnaire est terminé**
17. Où/comment avez-vous entendu parler de manque de sang ? _____
- Réponses dirigées : (01=oui, 02=non, 09=nsp)**
 a. centre de santé /_/ b. école /_/ c. campagne de sensibilisation /_/ d. mari /_/
 e. mère, grand mère /_/ f. sœur, ami(e) /_/ g. télévision /_/ h. radio /_/
 i. étude anémie j. autre (à préciser) : _____
18. On vous a dit quoi par rapport au manque de sang?

- Réponses dirigées : (01=oui, 02=non, 09=nsp)**
 a. maladie grave /_/ b. c'est bon /_/ c. maladie mystique /_/ d. maladie pas grave /_/

19. Le manque de sang, pour vous, c'est quoi ?

Réponses dirigées : (01=oui, 02=non, 09=nsp)
 a. la mort /___/ b. la sorcellerie /___/ c. la maladie /___/ d. la santé/___/ e. le bonheur /___/

II-1- ETIOLOGIE

20. Pour vous, qu'est ce qui donne l'anémie?
Réponses dirigées (01= oui ; 02=non ; 09=ne sait pas)
 a. Le soleil /___/ b. le paludisme (« djékouadjo »)/___/ c. les sorciers /___/ d. la grossesse /___/
 e. une alimentation qui n'est pas bonne /___/ f. des saignements abondants (ex : blessures, règles)/___/
 g. le feu /___/ h. Le non respect d'interdits /___/ i. Manque d'hygiène /___/ j. des vers dans le ventre/___/
 k. ictere /___/ l. La bilharziose (pisse du sang) /___/ m. le SIDA /___/
 n. des maladies héréditaires (l'enfant naît avec) /___/

II-2- SYMPTÔMES DE L'ANEMIE

21. Comment on sait que quelqu'un a l'anémie ?.....
Réponses dirigées : (01=oui, 02=non, 09=nsp)
 a. couleur de l'intérieur de l'œil (*la personne qui administre le questionnaire montre sur soi-même*) /___/
 b. couleurs des lignes dans la paume de la main (*la personne qui administre le questionnaire montre sur soi-même*) /___/
 c. couleurs de l'ongle (*la personne qui administre le questionnaire montre sur soi-même*) /___/
 d. pâleur du corps/___/ e. fatigue, somnolence /___/ f. faiblesse/___/ g. vertiges /___/
 h. cœur qui bat vite /___/ i. perte de poids/___/ j. il est gros/___/ k. il court vite/___/

II-3- CONSEQUENCES DE L'ANEMIE

22. Quelles peuvent être les conséquences d'une anémie ?

Réponses dirigées : (01=oui, 02=non, 09=nsp)
 a. fatigue, faiblesse /___/ b. difficultés à l'école (concentration, apprentissage...)/___/ c. la force/___/
 d. problèmes durant la grossesse ou à l'accouchement /___/
 e. maladies /___/ f. la mort /___/ g. la santé/___/

II-4- PERSONNES LES PLUS VULNERABLES

23. Quels sont les groupes qui souffrent le plus de ça
Réponses dirigées : (01=oui, 02=non, 09=nsp)
 a. enfants de moins de 5 ans /___/ b enfants d'âge scolaire /___/ c. jeunes hommes /___/
 d. jeunes femmes/___/ g. femmes enceintes /___/ f. vieillards /___/

III- EXPOSITION A L'ANEMIE

24. Est-ce que tu as déjà eu ça? (01=oui, 02=non; 09=nsp) /___/
Si la personne répond « NON », passez à la question 29

25. Si oui, combien de fois? /___/ fois

26. Comment as-tu su que tu avais l'anémie ?/___/ (01= propre jugement, 02=visite médicale, 03=tradipraticien, 04=information par un tiers, 04. autre, à préciser : _____)

27. Ca t'a donné quoi?.....
Réponses dirigées : (01=oui, 02=non, 09=nsp)
 a. fatigue /___/ b. force/___/ c. maladies /___/, précisez : _____
 d. difficultés à l'école/aux champs /___/ e. arrêt de travail/école /___/

28. Combien de temps l'anémie a-t-elle duré ? /___/ jours **OU /___/ mois **OU** /___/ années**

IV- PREVENTION DE L'ANEMIE	
29. Qu'est ce qu'on peut faire pour ne pas avoir l'anémie ?	
<i>Réponses dirigées : (01=oui, 02=non, 09=nsp)</i>	
a. Consultations prénatales /__/	b. Médecine traditionnelle /__/
d. Allaitement exclusif durant les 6 premiers mois de vie de l'enfant /__/	e. Prières, pratiques spirituelles /__/
f. Se protéger du paludisme /__/	
g. Objets de santé /__/	h. Faire un contrôle annuel à l'hôpital /__/
i. manger des tomates /__/	j. boire des sucreries /__/
c. Alimentation variée /__/	
V- ITINERAIRE THERAPEUTIQUE	
30. Qu'avez-vous fait lorsque vous étiez anémié ou que feriez-vous si cela vous arrivait ? _____	
Si la personne a répondu « NON » ou « ne sait pas » à la question 24, passez à la question 35.	
31. Avez-vous reçu un traitement lorsque vous étiez anémié? (01=oui, 02=non, 09=nsp) /__/	
Si la personne a répondu « NON » ou « ne sait pas », passez à la question 33	
32. Où avez-vous reçu un traitement ?	
a. Hôpital, centre de santé /__/	b. famille /__/
e. tradipraticien /__/	f. autre /__/
c. Pharmacie (dépôt) /__/	d. médicaments de la rue /__/.
33. Si possible, précisez le nom du traitement reçu: _____	
34. Qu'est ce qui a été plus efficace pour vous ? _____	
VI. HABITUDES ALIMENTAIRES	
35. Hier, tu as mangé quoi (du matin jusqu'au soir)?	
Matin : _____	
Midi : _____	
Soir : _____	
Autres : _____	
36. Est-ce qu'il y a des repas ou des boissons qu'on peut prendre pour ne pas avoir l'anémie ? (01=oui, 02=non, 09=nsp) /__/	
37. Si oui, lesquels ? _____	
38. Les aliments suivants sont-ils importants dans la prévention de l'anémie ? (01=oui, 02=non, 09=nsp)	
a. Viande, rognons /__/	b. Poisson /__/
c. oeuf /__/	d. sucreries
f. fruits (mangues, ananas, papayes, oranges, banane) /__/	g. kabato /__/
i. tomates (tomates) /__/	j. oignons, aubergines, choux /__/
e. attiéké /__/	h. bissap /__/
	k. le vin /__/
VI- INTERDITS ALIMENTAIRES	
39. Est-ce qu'il y a des repas/viande que tu ne DOIS pas manger? (01=oui, 02=non, 09=nsp) /__/	
40. Si oui, lesquels ? _____	
41. Pourquoi on t'a dit de ne pas manger ça? (01=oui, 02=non, 09=nsp)	
a. surveillance de la grossesse /__/	b. interdit familial ou communautaire /__/
d. Autre /__/, à préciser : _____	c. éviter des maladies /__/

☺☺☺☺☺☺ MERCI BEAUCOUP POUR TA PARTICIPATION !!! ☺☺☺☺☺☺

AS-TU DES QUESTIONS ?

10.3 Guide used for focus group discussions conducted with young women, school-aged children, village authorities, health staff and traditional healers.

DISCUSSION DE GROUPE FOCALISEE

Groupes : (i) jeunes filles ; (ii) enfants d'âge scolaire (7-9 ans)

I PERCEPTION DU SANG

1. Comment appelez-vous le sang dans votre ethnie ?
2. Que représente le sang dans votre communauté ?

II PERCEPTION DE L'ANEMIE (« manque de sang »)

II-1- Dénomination et représentations sociales

3. Comment appelez-vous le manque de sang dans votre ethnie ?
4. Que représente le manque de sang dans votre communauté ?

II-2- Etiologie sociale

5. Qu'est-ce qui provoque l'anémie ?
6. Qu'est-ce qui provoque le paludisme ?

II-3- Symptomatologie

7. Par quels signes/symptômes reconnaissez-vous celui qui est atteint d'anémie ?
8. Quels liens établissez-vous entre l'anémie et le poids du sujet anémié ?

II-4- Conséquences

9. Qu'est-ce que l'anémie peut provoquer ?

II-5 Prévention

10. Comment peut-on réduire les risques de développer une anémie ?

III HABITUDES ALIMENTAIRES

11. Quels sont les plats que vous consommez régulièrement dans votre ménage ?
12. Quels sont les aliments utiles à la prévention de l'anémie que vous connaissez ?

IV INTERDITS ALIMENTAIRES

13. Quels sont les interdits alimentaires chez les jeunes enfants dans votre communauté ?
14. Quels sont les interdits alimentaires chez les femmes dans votre communauté ?
15. Quels sont les interdits alimentaires chez les femmes enceintes dans votre communauté ?

DISCUSSION DE GROUPE FOCALISEE

Groupe : Notabilité

I PERCEPTION DU SANG

1. Comment appelez-vous le sang dans votre ethnie ?
2. Que représente le sang dans votre communauté ?

II PERCEPTION DE L'ANEMIE (« manque de sang »)

II-1- Dénomination et représentations sociales

3. Comment appelez-vous le manque de sang dans votre ethnie ?
4. Que représente le manque de sang dans votre communauté ?

II-2- Etiologie sociale

5. Qu'est-ce qui provoque l'anémie ?
6. Qu'est-ce qui provoque le paludisme ?

II-3- Symptomatologie

7. Par quels signes/symptômes reconnaissez-vous celui qui est atteint d'anémie ?
8. Quels liens établissez-vous entre l'anémie et le poids du sujet anémié ?

II-4- Conséquences

9. Qu'est-ce que l'anémie peut provoquer ?

II-5 Prévention

10. Comment peut-on réduire les risques de développer une anémie ?

III CALENDRIER DE PRODUCTION AGRICOLE

11. Quels sont les cultures agricoles qui existent dans votre village ?
12. A quel moment de l'année récolte-t-on chaque type de production agricole ?
(réalisation d'un calendrier selon modèle FAO ou selon modèle communal local)
13. Où se trouvent les différentes cultures par rapport au village ?

IV HABITUDES ALIMENTAIRES

14. Quels aliments consomme-t-on dans votre village ?
15. D'où ces cultures proviennent-elles ?

V INTERDITS ALIMENTAIRES

16. Quels sont les interdits alimentaires chez les jeunes enfants dans votre communauté ?
17. Quels sont les interdits alimentaires chez les femmes dans votre communauté ?
18. Quels sont les interdits alimentaires chez les femmes enceintes dans votre communauté ?

DISCUSSION DE GROUPE FOCALISEE/ ENTRETIENT INDIVIDUEL

Groupes : (i) personnel soignant de l'hôpital général de Taabo ; (ii) infirmier du centre de santé rural d'Ahondo

I AMPLEUR DE L'ANEMIE

1. Selon vous, quelle est l'ampleur de l'anémie dans la sous-préfecture de Taabo?
2. Données statistiques de la région ?

II ETIOLOGIE

3. Quelles en sont les causes majeures selon vous ?
4. Quelles en sont les caractéristiques ?

III SYMPTOMATOLOGIE

5. Par quels signes reconnaissez-vous celui qui est atteint d'anémie ?

IV DIAGNOSTIC

6. Quels sont les diagnostics à disposition des patients permettant de confirmer la présence d'une anémie ?
7. Quels sont les diagnostics à disposition des patients afin de déterminer la cause de l'anémie ?

V CONSEQUENCES

8. Qu'est-ce que l'anémie peut provoquer ?

VI PREVENTIONS

9. Quelles sont les mesures préventives relatives à l'anémie ?
10. Quels sont les conseils relatifs à la prévention de l'anémie que vous donnez aux populations?
11. Comment s'effectuent les activités de sensibilisation ?

ENTRETIEN INDIVIDUEL

Tradipraticien (praticien de la médecine de tradition africaine)

Questions 1-11 : voir questionnaire pour jeunes femmes et enfants

Question additionnelle : Avec quel médicament traitez-vous celui qui est anémié ?

