Aus dem Institut für Tropenmedizin und Internationale Gesundheit
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Vertikale HIV-Transmission im ländlichen Tansania: Herausforderungen bei der Umsetzung eines komplexen antiretroviralen Prophylaxeregimes

zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

von

Inga Lau

aus Leverkusen

Datum der Promotion: 25.10.2013

Inhaltsverzeichnis

Abkürzungsverzeichnis

ANC	Geburtsvorsorge (antenatal care)
ART	Antiretrovirale Therapie
ARV	Antiretrovirales Medikament (antiretroviral)
AZT	Zidovudin
CI	Konfidenzintervall (confidence interval)
HB	Hämoglobin
HIV	Humanes Immundefizienz-Virus (human immunodefiency virus)
IQR	Interquartilsabstand (interquartile range)
KDH	Distrikt-Krankenhaus von Kyela (Kyela District Hospital)
MCV	Durchschnittliches Erythrozytenvolumen (mean corposcular volume)
MTCT	Mutter-Kind-Übertragung von HIV (mother-to-child-transmission of HIV)
NNRTI	Nicht-nukleosidischer Reverse-Transkriptase-Inhibitor
NRTI	Nukleosidischer Reverse-Transkriptase-Inhibitor
NVP	Nevirapin
NVP-ED	Nevirapin-Einmaldosis
PCR	Polymerase-Kettenreaktion (polymerase chain reaction)
PMTCT	Prävention der Mutter-Kind-Übertragung von HIV (prevention of mother-to-child transmission of HIV)
RBC	Erythrozytenzahl (red blood cell count)
RDW	Erythrozytenverteilungsbreite (red cell distribution width)
RT	Reverse Transkriptase
SSW	Schwangerschaftswoche
WHO	Weltgesundheitsorganisation (World Health Organization)
3TC	Lamivudin

Zusammenfassung

1 Abstrakt

Weltweit werden jährlich 370.000 Kinder mit HIV infiziert. Der größte Teil von ihnen lebt in Subsahara-Afrika, und sie werden meist von ihren Müttern während Schwangerschaft, Geburt oder Stillzeit angesteckt. In Tansania wurden im Juni 2008 die Leitlinien zur Prävention der HIV-Mutter-Kind-Übertragung (prevention of mother-to-child-transmission of HIV, PMTCT) von einer Nevirapin-Einmaldosis zur Geburt auf ein antiretrovirales Kombinationsregime umgestellt. Mit Hilfe des Kombinationsregimes sollen Übertragungsraten gesenkt und Resistenzbildungen gegen Nevirapin vermindert werden. Wegen seiner Komplexität birgt dieses Regime allerdings die Gefahr, in infrastrukturell schwachen Regionen nicht optimal durchführbar zu sein. Insbesondere sind Herausforderungen bezüglich der Medikamentenadhärenz, komplexer Resistenzbildungen und toxischer Medikamentennebenwirkungen zu befürchten. Ziel dieser Arbeit ist es, die Umsetzung des komplexen Medikamentenregimes zur PMTCT unter realen Bedingungen in einer peripheren Region Tansanias zu evaluieren.

Dafür wurde im Distrikt-Krankenhaus von Kyela an 184 HIV-positiven Schwangeren eine Beobachtungsstudie durchgeführt, die sich in mehrere Substudien untergliederte. In Substudie 1 wurde die Medikamentenadhärenz mit Hilfe eigenständig erstellter Fragebögen analysiert. Diese stellte sich insgesamt als schwach heraus: Nur ein Mutter-Kind-Paar erreichte ein Adhärenzniveau von ≥95% für alle Phasen der Prophylaxeintervention. Ein frühes Gestationsalter bei Erstvorstellung in der Schwangerschaftsvorsorge stellte einen Risikofaktor dar, in der Schwangerschaft nicht mit einer Medikamenteneinnahme zu beginnen. Die HIV-Statusoffenbarung hingegen stand im Zusammenhang mit höheren vorgeburtlichen Adhärenzwerten. Substudie 2 untersuchte das Auftreten von Resistenzen gegen die eingesetzten Medikamente. Dafür wurden Allelspezifische Polymerasekettenreaktionen durchgeführt, mit denen für 40% der Mütter resistente Virusvarianten nachgewiesen werden konnten. Die Einnahme einer mehr als zehnwöchigen Monoprophylaxe mit Zidovudin (AZT) während der Schwangerschaft war mit einem erhöhten Auftreten von AZT-Resistenzen zur Geburt assoziiert. Die in Substudie 3 beobachteten medikamenteninduzierten Blutbildveränderungen waren transient und meist mild. Jedoch wiesen einige wenige Kinder Fälle schwerer Hämatotoxizität auf.

Insgesamt stellte die Umsetzung des komplexen Prophylaxeregimes in dieser infrastrukturell schwachen Region Tansanias eine große Herausforderung dar, sowohl für die betroffenen Frauen, als auch für das Gesundheitspersonal. Zur Steigerung der vorgeburtlichen Adhärenz wären eine stärkere Unterstützung der betroffenen Frauen durch enge Vertrauenspersonen und eine

intensivere Personalschulung wünschenswert. Um die Fälle der frühen Prophylaxeablehnung zu verringern, hat Tansania bereits einen früheren AZT-Prophylaxebeginn in der Schwangerschaft eingeführt. Die längere vorgeburtliche AZT-Einnahme erhöht jedoch die Gefahr von AZT-Resistenzbildungen. Um diesen vorzubeugen, erscheint der Einsatz einer Medikamentenkombination bereits zum Prophylaxebeginn sinnvoll. Gleichzeitig wäre die Einführung routinemäßiger Blutbildkontrollen für AZT-haltige PMTCT-Interventionen erstrebenswert, um auftretende Fälle schwerer Hämatotoxizität nicht zu übersehen.

2 Abstract

Approximately 370,000 children are infected every year with the human immunodeficiency virus (HIV). Most of them are living in sub-Saharan Africa, where they are usually infected by their mothers during pregnancy, delivery or breastfeeding. In June 2008, Tanzania changed its guidelines for the prevention of mother-to-child-transmission (PMTCT) of HIV to a combination medication regimen, in an effort to lower transmission rates and to curb the development of drug-resistance against the formerly recommended nevirapine single-dose. However, the feasibility of this new regimen in areas of limited resources is questionable due to concerns regarding adherence, the subsequent complex drug-resistance, and toxic side effects. This thesis aims to evaluate the implementation of the combination prophylaxis regimen under real-life conditions in rural Tanzania.

In the Kyela District Hospital, 184 HIV-positive pregnant women were enrolled in this observational study, which was further divided into several substudies. Substudy 1 analyzed adherence to the combination prophylaxis using standardized questionnaires. Ultimately, only one mother-child-pair could maintain at least 95% adherence throughout the prophylactic intervention. An early gestational stage at the first prenatal care visit was identified as a risk-factor for not starting pre-delivery prophylaxis, while HIV-status disclosure to a person of trust was related to higher adherence rates during the gestational period. The emergence of resistance against antiretroviral drugs was quantified in Substudy 2. Allele-specific polymerase chain reactions were used to detect resistant viral strains in 40% of the women. The prenatal intake of zidovudine (AZT) monoprophylaxis for more than ten weeks was associated with increased emergence of AZT-resistance at the time of delivery. Substudy 3 monitored blood counts, showing mostly mild and transient changes for the mothers, while few children presented cases of severe haematological toxicity.

Overall, the implementation of the combination prophylaxis regimen in rural Tanzania has shown to be a major challenge for the affected mothers, as well as for healthcare workers. Adherence rates could be drastically improved by enhancing the provision of social support to the

affected women, as well as scaling up PMTCT training for staff. In an effort to minimize cases of rejection of prophylaxis, Tanzania has introduced an early prophylaxis start during pregnancy. Unfortunately, a prolonged AZT-intake during pregnancy increases the risk of AZT-resistance, so that the use of a drug combination early in the regimen would be advisable. At the same time, monitoring blood counts should be implemented as a constituent part of any PMTCT services using AZT-containing regimen in order not to oversee cases of severe haematological toxicity.

3 Einleitung

Weltweit sind über zwei Millionen Kinder unter 15 Jahren mit dem Humanen Immundefizienz-Virus (HIV) infiziert und jährlich werden 370.000 Kinder neu angesteckt. Die Übertragung von HIV auf Kinder findet hauptsächlich durch vertikale Mutter-Kind-Transmission (mother-to-child-transmission of HIV, MTCT) während Schwangerschaft, Geburt oder Stillzeit statt. Am schwersten ist das ressourcenschwache Subsahara-Afrika von der Epidemie betroffen[1]. Dort besteht ohne Intervention ein vertikales Transmissionsrisiko von 25-48%[2].

Neben einem in ressourcenschwachen Regionen meist nicht durchführbaren Stillverzicht werden zur Prävention der Mutter-Kind-Übertragung von HIV (prevention of mother-to-child-transmission of HIV, PMTCT) hauptsächlich antiretrovirale Medikamente (ARVs) eingesetzt. Durch die Einnahme von Nevirapin (NVP), einem nicht-nukleosidischen Reverse-Transkriptase-Inhibitor (NNRTI), kann eine Senkung der vertikalen Transmission auf unter 15% erreicht werden[3]. Die einmalige Einnahme des kostengünstigen NVP zur Geburt (NVP-ED) wurde nicht zuletzt wegen ihrer einfachen Anwendbarkeit in ressourcenlimitierten Regionen lange als PMTCT-Standard favorisiert. Allerdings konnten nach NVP-ED eine hohe Rate an Resistenzen gegen NVP und andere NNRTIs beobachtet werden[4]. Um der Bildung von NVP-selektierten Resistenzen vorzubeugen und das Risiko der vertikalen HIV-Transmission weiter zu reduzieren, empfiehlt die Weltgesundheitsorganisation (WHO) seit 2006 unter anderem ein Kombinationsprophylaxeregime aus NVP und zwei nukleosidischen Reverse-Transkriptase-Inhibitoren (NRTIs), Zidovudin (AZT) und Lamivudin (3TC)[5].

Den WHO-Leitlinien von 2006 folgend wurden 2008 auch in Tansania, einem der ärmsten und am wenigsten entwickelten Länder der Welt[6], neue PMTCT-Leitlinien eingeführt: Allen HIV-positiven schwangeren Frauen, die keiner antiretroviralen Therapie (ART) bedürfen, sollten ab der 28. Schwangerschaftswoche (SSW) 2x täglich 300mg AZT als medikamentöse Prophylaxe angeboten werden. Zur Geburt waren die einmalige Gabe von 200mg NVP, die dreistündliche Gabe von 300mg AZT und die zwölfstündliche Gabe von 150mg 3TC vorgesehen. Die Einnahme von je 2x täglich 300mg AZT und 150mg 3TC sollte postpartal für eine Woche fortgesetzt werden. Alle Kinder sollten zur Geburt 2mg/kg NVP und, abhängig von der Dauer der mütter-

lichen AZT-Einnahme während der Schwangerschaft, postpartal für eine oder vier Wochen AZT erhalten. NVP-ED blieb als Mindeststandard dem Ausnahmefall vorbehalten[7].

Eine optimale Adhärenz ist für die Wirksamkeit der antiretroviralen Prophylaxe unerlässlich. Bei einer regelmäßigen Medikamenteneinnahme wird die mütterliche Viruslast deutlich gesenkt[8], und somit der größte Risikofaktor für eine vertikale HIV-Transmission[9] minimiert. Darüber hinaus birgt eine unzureichende Adhärenz trotz Medikamentenkombination die Gefahr der Entstehung resistenter HIV-Varianten, die konsekutiv zu einem Versagen einer sich anschließenden ART führen können[10]. Die Überwachung der Medikamentenadhärenz der Mütter ist wegen der möglichen Übertragung von resistenten Virusvarianten auf ihre Kinder besonders wichtig. Bisher wurde die Adhärenz zu komplexen PMTCT-Regimen in peripheren und infrastrukturell schwachen Regionen zwar erst wenig untersucht, jedoch lässt die schwache Akzeptanz einfacher Prophylaxeregime wie NVP-ED[11] für komplexe Prophylaxeregime in ländlichen afrikanischen Regionen eine suboptimale Adhärenz befürchten.

Zusätzlich birgt die während der Schwangerschaft vorgesehene mehrwöchige Monoprophylaxe mit AZT, vor allem bei suboptimaler Adhärenz, das Risiko einer Selektion für AZT-resistente Viren[12]. Da sowohl NVP als auch 3TC eine niedrige genetische Barriere haben und somit schnell Resistenzen hervorrufen, ist auch die Entstehung multiresistenter HIV-Varianten denkbar. Selbst zu einem geringen Anteil in der Gesamtpopulation eines Patienten vorliegende, d. h. minoritäre resistente Virusvarianten, können einen späteren Therapieerfolg beeinträchtigen[13,14]. Bislang gibt es allerdings keine Resistenzstudien mit Betrachtung minoritärer Virusvarianten für die seit 2006 empfohlene ARV-Kombinationsprophylaxe.

Gerade in peripheren und ressourcenschwachen Regionen sind teils weder regelmäßige klinische Kontrollen noch adäquate Behandlungsmöglichkeiten möglicher toxischer Medikamentennebenwirkungen gegeben. In diesen Gebieten ist es daher angeraten, das Nebenwirkungsrisiko eingesetzter Medikamente besonders gut zu kennen. Das im Rahmen der antiretroviralen Kombinationsprophylaxe am längsten verabreichte Medikament, AZT, kann Blutbildveränderungen wie Anämie und Granulozytopenie hervorrufen[15,16]. Um diese potentiellen hämatotoxischen Nebenwirkungen adäquat zu überwachen, sind engmaschige Blutbildkontrollen bei Müttern und Kindern erforderlich.

Bisher wurden Studien zu komplexen PMTCT-Regimen in ressourcenschwachen Ländern häufig in dortigen Metropolen durchgeführt[17]. Da jedoch die Mehrheit der tansanischen Bevölkerung in ländlicher Umgebung lebt[18], ist es wichtig, Adhärenz und weitere Einflussfaktoren auf die Umsetzbarkeit eines solchen Regimes unter realistischen Bedingungen in einem peripheren Setting zu betrachten.

4 Zielstellung

Die Zielsetzung der Studie war eine Evaluation der Umsetzung der tansanischen PMTCT-Leitlinien von 2008 an einem exemplarischen, ländlich gelegenen Krankenhaus. Mögliche Herausforderungen bezüglich Medikamentenadhärenz, Resistenzbildungen und Nebenwirkungen sollten dort unter realen Bedingungen untersucht werden.

- Ziel der ersten Substudie war es, die Medikamentenadhärenz der betroffenen Frauen zu dem komplexen Kombinationsregime zu bestimmen und Einflussfaktoren auf die Adhärenz zu eruieren (Publikation 1).
- Ziel der zweiten Substudie war es, die Entstehung resistenter HIV-1-Varianten gegen AZT, NVP und/oder 3TC inklusiver minoritärer Virusvarianten nach Einnahme der komplexen Kombinationsprophylaxe nachzuweisen und zu quantifizieren (Publikation 2).
- Ziel der dritten Substudie war es, potentiell toxische Auswirkungen der AZT-haltigen Kombinationsprophylaxe auf das Blutbild der HIV-positiven Mütter und ihrer Kinder zu untersuchen (Publikation 3).

Die Studienergebnisse sollen dazu beitragen, die tansanischen PMTCT-Leitlinien optimal an die örtlichen Gegebenheiten anzupassen und so die vertikale Transmission von HIV in Tansania bestmöglich zu reduzieren.

5 Methodik

Setting: Die Beobachtungsstudie wurde im Distrikt-Krankenhaus von Kyela (KDH) durchgeführt, einer peripher gelegenen Gesundheitseinrichtung der Region Mbeya, Tansania. Sie war dort in ein vom Bundesministerium für wirtschaftliche Zusammenarbeit und Entwicklung durch die Gesellschaft für Internationale Zusammenarbeit (GIZ) gefördertes PMTCT-Programm eingebettet[19]. Alle dieser Doktorarbeit zugrunde liegenden Daten stammen von einer Kohorte HIV-positiver schwangerer Frauen, die sich nach erfolgter Umstellung von NVP-ED auf das komplexe ARV-Prophylaxeregime zur Geburtsvorsorge (antenatal care, ANC) oder zur Entbindung im KDH zwischen Oktober 2008 und September 2009 vorstellten.

Routineablauf im KDH: Eine HIV-Beratung und ein freiwilliger HIV-Test fanden im KDH routinemäßig zur Erstvorstellung, entweder in der ANC oder auf der Entbindungsstation statt. Bei positivem HIV-Status wurde, abhängig von CD4-Zellzahl und klinischem Erscheinungsbild gemäß WHO-Definition[20], zwischen Therapie- und Prophylaxeindikation unterschieden. Eine Therapieindikation bestand laut Leitlinien bei einer CD4-Zellzahl von <200/µl bei klinischen HIV-Infektionsstadien 1 und 2, einer CD4-Zellzahl von <350/µl bei klinischem Stadium 3 oder bei klinischem Stadium 4, unabhängig von der CD4-Zellzahl. Allen anderen Frauen wurde eine

ARV-Kombinationsprophylaxe angeboten, sofern keine schwere Anämie (Hämoglobin (HB) <7,5g/dl) oder Granulozytopenie (<0,75/nl) vorlag. Die ARV-Prophylaxe wurde entsprechend den tansanischen PMTCT-Leitlinien von 2008 verabreicht, die ein dreiphasiges Prophylaxeregime vorsahen: während der Schwangerschaft, unter der Geburt und postpartal[7]. Die benötigten AZT-Dosen wurden in den ersten vier Wochen nach Prophylaxebeginn (ab SSW 28 oder später) für jeweils 7 Tage an die Frauen ausgegeben, danach für jeweils 30 Tage. Alle Frauen wurden dazu angehalten, im KDH zu entbinden; unter der Geburt wurden dann NVP, AZT und 3TC innerhalb der Entbindungsstation des KDH verabreicht. Die postpartale Prophylaxephase begann im Krankenhaus und wurde bei Entlassung zu Hause bis zu vier Wochen fortgesetzt. Für den Fall einer Geburt außerhalb des KDH erhielten alle PMTCT Patientinnen ab SSW 28 eine NVP-Tablette, um diese bei Einsetzen der Geburtswehen selbstständig einzunehmen. In diesem Fall sollten sie sich innerhalb von 72 Stunden nach Geburt mit ihrem Kind im KDH vorstellen, um die NVP-ED für ihr Kind und die postpartalen Prophylaxemedikamente für sich und das Kind abzuholen. Nach der Geburt waren Kontrollbesuche in den Wochen 1-2 und 4-6 vorgesehen.

Studienprotokoll: Für unsere Studie galten das nicht erreichte Mindestalter von 18 Jahren und bekannte psychische Probleme als zusätzliche Ausschlusskriterien zu den von den Leitlinien vorgegebenen Kontraindikationen gegen die ARV-Kombinationsprophylaxe (Anämie, Granulozytopenie und ART-Indikation). Nach eingehender Aufklärung über den Studienablauf und schriftlicher Einverständniserklärung wurden alle Frauen in unsere Studie aufgenommen, die keinerlei Ausschlusskriterien aufwiesen. Jede Teilnehmerin konnte ihr Einverständnis jederzeit widerrufen und das Studienprotokoll verlassen.

Zur Erhebung von soziodemografischen, -ökonomischen und klinischen Parametern wurden standardisierte und strukturierte Fragebögen erstellt. Zu jedem ANC-Besuch wurden Adhärenz, Gründe für Nicht-Adhärenz, HIV-Statusoffenbarung gegenüber einer Vertrauensperson und zusätzlich eingenommene Medikamente abgefragt. Auf der Entbindungsstation wurde zusätzlich ein Fragebogen mit Informationen über Geburtsverlauf, Medikamenteneinnahme und Kindesdaten von der jeweilig betreuenden Hebamme ausgefüllt.

Blutabnahmen fanden zu jedem Vorstellungstermin vor der Geburt, einmalig unter der Geburt und in den Wochen 1-2, 4-6 und 12-16 nach der Geburt statt. Unter der Geburt wurde Nabelschnurblut gewonnen, und falls möglich wurde den Kindern zusätzlich in Woche 4-6 und in Woche 12-16 nach Geburt Blut abgenommen. Aliquots der Plasmaproben wurden bei -20°C tiefgefroren und nach Deutschland gesandt.

Die Studie wurde mit Genehmigung des *National Institute for Medical Research Tanzania*, des *Mbeya Ethical Research Committees* und der Ethikkommission der Charité-Universitätsmedizin Berlin durchgeführt. Die Daten wurden anonymisiert und streng vertraulich behandelt.

5.1 Substudie 1: Adhärenz zur MTCT-Kombinationsprophylaxe in Tansania

Subkohorte 1: Die Medikamentenadhärenz wurde für alle Frauen (und deren Kinder) bestimmt, die über die ANC in die Studie aufgenommen wurden, unabhängig von ihrem Entbindungsort. Studienteilnehmerinnen, die nicht im KDH entbunden hatten und sich nicht innerhalb von 72 Stunden nach Geburt dort vorstellten, galten als *lost to follow-up*.

Adhärenzdefinition: Während der Schwangerschaft wurde die Medikamentenadhärenz als *Medication-Possession-Ratio* definiert, das heißt als Verhältnis zwischen den im Krankenhaus ausgegebenen AZT-Dosen und den zwischen Prophylaxebeginn und Geburt theoretisch benötigten Dosen. 100% Adhärenz entsprach daher einer termingerechten Abholung aller vorgesehenen Medikamentendosen. Die Bestimmung der Medikamentenadhärenz für Mütter und Kinder unter der Geburt und postpartal basierte auf den von den Hebammen notierten Ausgaben der jeweiligen Medikamentendosen. Unter der Geburt wurde sie für NVP als Anteil aller korrekt ausgegebenen Dosen und für AZT und 3TC als Anteil der leitliniengemäß abgedeckten Stunden definiert. Postpartal setzte sie sich für den Zeitraum des stationären Aufenthaltes aus den korrekt abgedeckten Stunden und den korrekt ausgegebenen Dosen zur Mitnahme nach Hause im Verhältnis zu den vorgesehenen Dosen zusammen.

Statistik: Für die statistische Auswertung wurde das Programm PASW 18 genutzt. Für die basisdemografischen Daten wurden Mediane berechnet und der gesamte Wertebereich angegeben. Die Unabhängigkeit kategorischer Daten wurde mit χ^2 und exaktem Fischer-Test überprüft. Einflüsse soziodemografischer Variablen auf den Beginn der Prophylaxeintervention wurden mit univariaten Odds Ratios untersucht; signifikante Faktoren ($p<0,05$) wurden in ein logistisches Regressionsmodell zur Berechnung adjustierter Odds integriert. Adhärenzwerte wurden für alle Phasen der medikamentösen Prophylaxe ermittelt und 80% bzw. 95% Adhärenz als kritische Grenzwerte festgelegt. Der Einfluss soziodemografischer Variablen auf die Adhärenzwerte wurde mit Hilfe des Mann-Whitney-U-Tests überprüft.

5.2 Substudie 2: Auftreten von medikamentenresistenten HIV-Varianten nach Einnahme einer komplexen antiretroviralen MTCT-Prophylaxe in Tansania

Subkohorte 2: In der Resistenzanalyse wurden die Frauen betrachtet, die für mindestens 2 Wochen AZT bekommen und NVP zu Beginn der Geburtswehen eingenommen hatten. Darüber hinaus sollten Plasmaproben von der Geburt und mindestens zwei unterschiedlichen postpartalen Kontrollterminen vorhanden sein. Die Kinder dieser Frauen wurden in die Analyse einbezogen, falls in Woche 4-6 (oder früher) eine HIV-Infektion nachgewiesen werden konnte.

Labormethoden: Alle betrachteten Mutationen liegen in dem Abschnitt des *pol* Gens, der die Reverse Transkriptase (RT) kodiert, und können per Allel-spezifischer Polymerase-Kettenreaktion (ASPCR) ab einer Häufigkeit von <1% nachgewiesen werden. Die jeweiligen Proben wurden auf drei wichtige AZT-selektierte Mutationen (K70R, T215Y, und T215F), zwei der häufigsten Mutationen bei NVP (K103N und Y181C) und die häufigste 3TC-selektierte Mutation M184V untersucht[21]. Für jede Plasmaprobe wurde eine äußere Polymerase-Kettenreaktion (PCR) in Echtzeit durchgeführt, um die Viruslast zu bestimmen und das benötigte RT-Fragment zu amplifizieren. Auf diesem Fragment konnten in jeweils einem weiteren ASPCR-Essay ggf. Resistenzmutationen nachgewiesen werden. Zur Bestimmung des HIV-Status der Kinder wurde eine RT-PCR mit Plasma aus Woche 4-6 durchgeführt.

Statistik: Die Statistische Auswertung wurde mit PASW 18 durchgeführt. Zur deskriptiven Analyse wurden Mediane berechnet und der jeweilige Interquartilsabstand (IQR) angegeben. Signifikante Unterschiede zwischen unabhängigen Proben wurden mit Hilfe des Mann-Whitney-U-Tests bestimmt, wohingegen Wiederholungsmessungen mit dem Wilcoxon-Vorzeichen-Rang-Test analysiert wurden. Die Unabhängigkeit kategorischer Daten wurde mit χ^2 und exaktem Fischer-Test überprüft. Zum Nachweis signifikanter Korrelationen zwischen kontinuierlichen Variablen ($p<0,05$) wurde Pearsons Korrelationskoeffizient genutzt.

5.3 Substudie 3: Hämatologische Veränderungen bei Frauen und Kindern, die einem AZT-haltigen Regime zur PMTCT in Tansania ausgesetzt waren

Subkohorte 3: Zur Hämatotoxizitätsanalyse wurden die Frauen, die in der ANC in die Studie aufgenommen worden waren und für mindestens eine Woche AZT eingenommen hatten, Gruppe 1 zugeordnet, und die auf der Entbindungsstation aufgenommenen Frauen Gruppe 2. Die Kinder der Gruppe-1- und Gruppe-2-Frauen wurden in die Analyse mit einbezogen, wenn sie entsprechend der Leitlinien AZT für eine Woche (Gruppe-1-Kinder) oder für vier Wochen (Gruppe-2-Kinder) erhalten hatten. HIV-positiv getestete Kinder wurden aus der Analyse ausgeschlossen, da HIV selbst Blutbildveränderungen hervorrufen kann.

Labormethoden: Von jeder im KDH gewonnenen Blutprobe wurde vor Ort ein großes Blutbild angefertigt. Die Bestimmung des HIV-Status der Kinder zur Geburt und in Woche 4-6 fand per RT-PCR statt.

Statistik: Die statistische Auswertung wurde mit Stata Version 11 durchgeführt. Die basisdemografischen Angaben wurden als Mediane mit gesamtem Wertebereich dargestellt und für beide Gruppen per T-Test, Mann-Whitney-U-Test oder exaktem Fischer-Test verglichen. Mit Hilfe des Mann-Whitney-U-Tests und des T-Tests wurden signifikante Unterschiede zwischen hämatologischen Werten zwischen den Gruppen zur Geburt, Monat 1 und Monat 3 getestet. Als Signifi-

kanzniveau wurde p=0.05 festgelegt. Die Infektionsraten der Kinder in Woche 6 wurden nach Kaplan-Meier geschätzt. Als Definition hämatotoxischer Blutbildveränderungen galten altersadjustierte Hämoglobinwerte von ≤10g/dl als leichte bzw. ≤7,4g/dl als schwere Anämie und Granulozytenzahlen von ≤5/nl als leichte bzw. ≤3/nl als schwere Granulozytopenie.

6 Ergebnisse

Von 1935 in ANC bezüglich einer HIV-Infektion getesteten Frauen bestätigte sich für 202 (14,5%) ein positives Testergebnis. Auf 184 Frauen trafen alle Einschlusskriterien zu. 122 Frauen wurden in ANC in die Studienkohorte aufgenommen, von denen 87 Frauen (71,3%) mit einer AZT-Prophylaxe begannen. 62 Frauen wurden auf der Entbindungsstation HIV-positiv getestet und willigten dort in die Studienteilnahme ein.

6.1 Substudie 1: Adhärenz zur MTCT-Kombinationsprophylaxe in Tansania

Kohorte: Die 122 Kohortenteilnehmerinnen wiesen zum Zeitpunkt der Aufnahme in die Studie im Median folgende Charakteristika auf: Alter 26 Jahre (Wertebereich 18-37), Gestationsalter 23 SSW (9-36), Schulzeit 7 Jahre (0-12), Fahrtzeit zum Krankenhaus 30 Minuten (0-120), Transportkosten 0 Tansanische Schilling (0-2000), CD4-Zellzahl 391/µl (200-883). 7 Frauen (5,7%) wurden gemeinsam mit ihrem Partner zur HIV-Infektion beraten und HIV-getestet.

Gesamtadhärenz: Ursprünglich hatten sich 122 Frauen bereit erklärt, die antiretrovirale Kombinationsprophylaxe leitliniengetreu einzunehmen. Von diesen erlitt eine Frau eine Fehlgeburt; ein Kind starb kurz nach der Geburt. Von den verbleibenden 120 Mutter-Kind-Paaren erreichten 10 Paare (8,3%) mindestens 80% Adhärenz; nur ein Mutter-Kind-Paar erreichte 95% Adhärenz für die gesamte PMTCT-Intervention.

AZT-Adhärenz während der Schwangerschaft: Von den 122 Frauen begannen 35 (28,7%) trotz vorheriger Einwilligung keine AZT-Prophylaxe während der Schwangerschaft. In der univariaten und multivariaten Regressionsanalyse zeigten sich ein Alter der Mutter von <23 Jahren (p<0,001; p=0,002), das Fehlen von eigenem Einkommen (p=0,006; p=0,015) und ein Gestationsalter von <24½ Wochen bei Erstvorstellung in der ANC (p<0,001; p=0,001) als signifikante Einflussfaktoren darauf, dass mit der vorgeburtlichen AZT-Prophylaxe nicht begonnen wurde. 11 dieser 35 Frauen entbanden trotzdem im KDH.
Von den 87 Frauen, die mindestens einmal AZT im KDH abgeholt hatten, hatte eine Frau eine Fehlgeburt und wurde aus der weiteren Analyse ausgeschlossen. Die übrigen 86 Frauen erreichten im Median eine vorgeburtliche AZT-Adhärenz von 77%. 56 Frauen (65,1%) erreichten ein Adhärenzniveau von mindestens 80% und 43 Frauen (50,0%) ein Adhärenzniveau von mindestens 95% (vgl. Abbildung 1). 33 Frauen (38,4%) waren in dieser Phase 100% adhärent, d.h. hol-

ten alle vor der Geburt vorgesehenen AZT-Dosen im KDH ab. Von den 86 Frauen offenbarten 6 (7%) ihren HIV-Status nicht. Die Status-Geheimhaltung war als einziger Parameter mit einem Adhärenzmedian von 22,7% signifikant mit einer schlechteren Adhärenz assoziiert als eine Statusoffenbarung (Median 97,3%; p=0,004).

53 der 86 Frauen (61,6%) holten mindestens eine Medikamentendosis verspätet oder gar nicht ab. Von diesen 53 Frauen gaben 19 Frauen insgesamt 24 Gründe für die versäumte Medikamentenabholung an. Am häufigsten wurden persönliche Gründe genannt (14x), unter anderem familiäre Verpflichtungen, vergessene Termine und Transportschwierigkeiten. 10 der genannten Gründe waren gesundheitspersonalbedingt und beinhalteten beispielsweise falsche Terminangaben und die Verweigerung der Medikamentenabgabe durch das Personal.

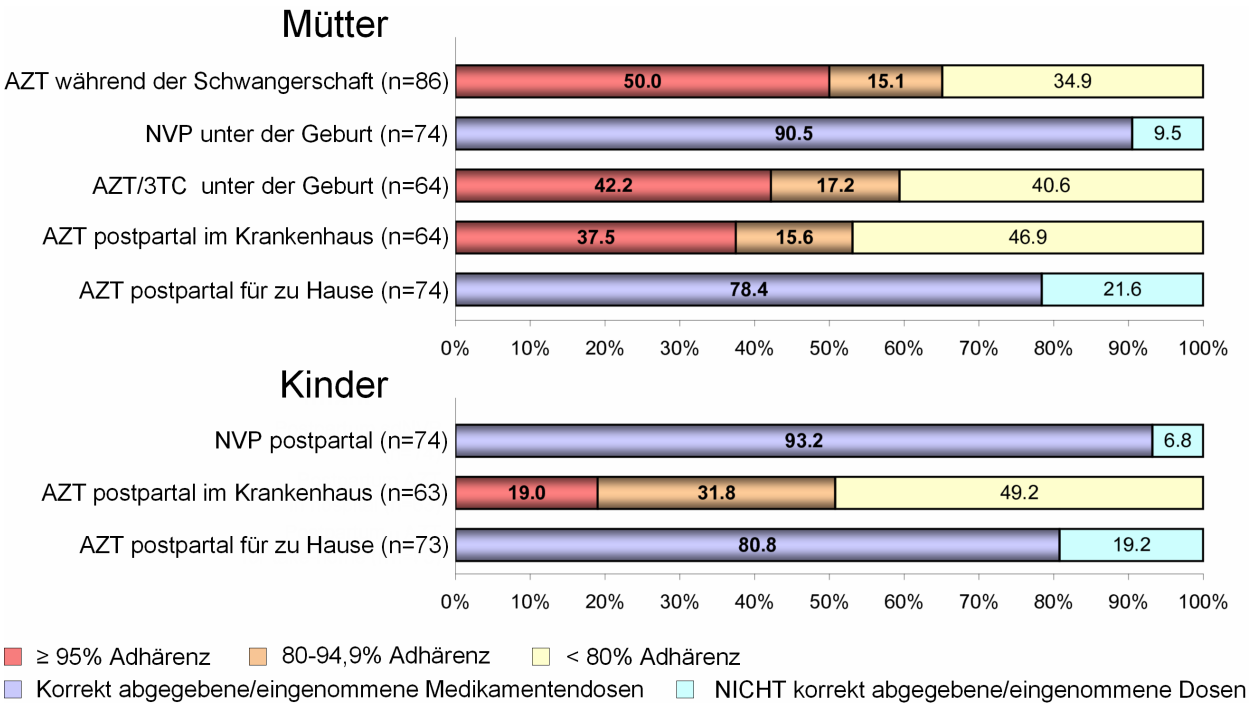

Abbildung 1: Prozentuale Darstellung von Müttern und Kindern, die während der unterschiedlichen Prophylaxephasen Adhärenzniveaus von ≥95% und ≥80% erreichten oder die vorgesehenen Medikamentendosen erhielten bzw. einnahmen.

Adhärenzniveaus unter der Geburt und postpartal: Für 74 Frauen der 122 Kohortenteilnehmerinnen konnte die Adhärenz unter und nach der Geburt bestimmt werden. 64 Frauen (86,5%) entbanden im KDH, während 10 (13,5%) innerhalb von 72 Stunden nach einer Hausgeburt zur Abholung der Prophylaxemedikamente in das KDH kamen. Die Vorstellung im KDH bis spätestens 72 Stunden nach der Geburt war signifikant mit der Einnahme der AZT-Prophylaxe während der Schwangerschaft assoziiert (p<0,001). Unter der Geburt im KDH erzielten 42,2% der Mütter ein 95%iges Adhärenzniveau. Nach der Geburt erreichten 37,5% der vorstelligen Mütter und 19% der Kinder ≥95% Adhärenz. 78,4% der Frauen erhielten laut Auf-

zeichnung durch das Gesundheitspersonal die korrekte Anzahl AZT-Dosen zur Mitnahme nach Hause. Von ihren Kindern wurden 93,2% postpartal mit NVP und 80,8% mit der korrekten AZT-Menge für zu Hause versorgt. Die erreichten Adhärenzniveaus von Müttern und Kindern sind in Abbildung 1 für alle Phasen der antiretroviralen Kombinationsprophylaxe dargestellt.

6.2 Substudie 2: Auftreten von medikamentenresistenten HIV-Varianten nach Einnahme einer komplexen antiretroviralen MTCT-Prophylaxe in Tansania

Kohorte: In der Resistenzanalyse wurden Blutproben von 50 Frauen und ihren insgesamt 7 HIV-positiven Kindern untersucht. Die Frauen wiesen zur Aufnahme in die Studie im Median eine HI-Viruslast von $1{,}25 \cdot 10^4$ Kopien/ml (IQR $4{,}4 \cdot 10^3$-$4{,}5 \cdot 10^4$) und eine CD4-Zellzahl von 390/µl (IQR 260-492) auf. In keiner der Ausgangsproben waren Resistenzmutationen der RT nachweisbar. Die mediane vorgeburtliche AZT-Einnahmedauer betrug 53 Tage (IQR 39-74).

Resistenzen der Mütter: Insgesamt konnte in den Plasmaproben von 20 der 50 untersuchten Frauen Virusvarianten mit RT-Resistenzmutationen nachgewiesen werden. Die Verteilung der Resistenzkombinationen ist in Abbildung 2 dargestellt. Mutationen, die zu einer verminderten Wirksamkeit von AZT führen, wurde im HIV-Genom bei insgesamt 11 infizierten Frauen gefunden (22%), davon wiesen die resistenten Virusvarianten in 7 Fällen eine alleinige K70R Mutation und in 4 Fällen zusätzlich eine T215Y/F Mutation auf. Virusvarianten, die bei 9 Frauen (18%) gefunden wurden, zeigten Resistenzen gegen NVP (K103 und/oder Y181C). Bei 4 Frauen (8%) wurden 3TC-resistente Virusvarianten (M184V) nachgewiesen, in 3 Fällen in Kombination mit Resistenzen gegen weitere antiretrovirale Medikamente. 5 Frauen wiesen bereits zur Geburt AZT-resistente HIV-Varianten auf. Dabei waren signifikant häufiger Frauen betroffen, die während der Schwangerschaft für 10 Wochen oder länger AZT eingenommen hatten (3/10=33%), als Frauen, die AZT für weniger als 10 Wochen bekommen hatten (2/40=5%; p=0,048). Bei 14 der 20 mit resistenten HI-Viren infizierten Frauen (70%), wurden ausschließlich minoritäre resistente Virusvarianten mit einer Häufigkeit <5% der HIV-Gesamtpopulation nachgewiesen.

Resistenzen der Kinder: In Woche 4-6 waren 7 von 49 Kindern HIV-infiziert, was einer Transmissionsrate von 14,3% entspricht. Bei 3 der 7 Kinder wurden resistente Virusvarianten gefunden. In allen 3 Fällen wiesen die HI-Viren Resistenzen gegen NVP auf, die bei den jeweiligen Müttern nicht nachgewiesen werden konnten. Eines dieser Kinder hatte postpartal weder NVP noch AZT bekommen. Ein anderes Kind, das die postpartalen Prophylaxemedikamente leitliniengerecht erhalten hatte, wurden zusätzlich AZT-resistente HI-Viren nachgewiesen, die auch bei der Mutter gefunden worden waren.

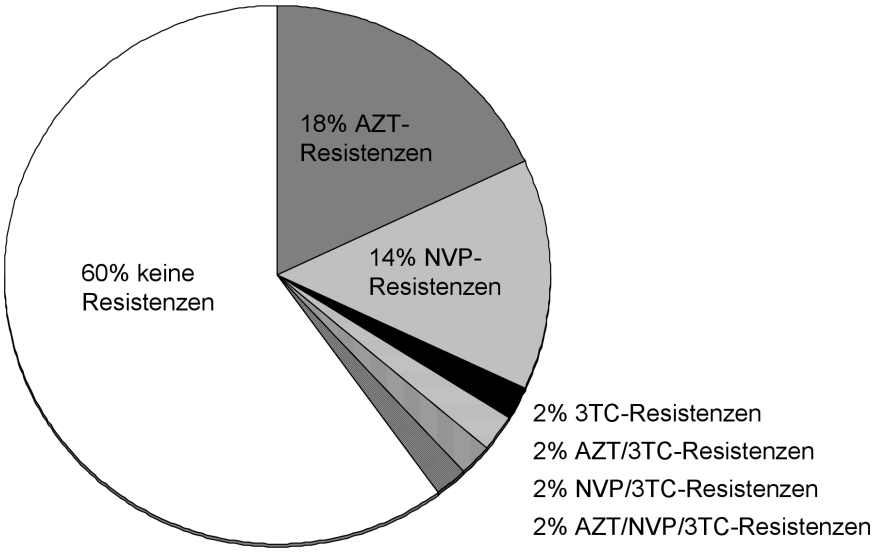

Abbildung 2: Verteilung der ARV-resistenten HIV-Varianten bei 50 Tansanischen Schwangeren nach Einnahme der antiretroviralen Kombinationsprophylaxe.

6.3 Substudie 3: Hämatologische Veränderungen bei Frauen und Kindern, die einem AZT-haltigen Regime zur PMTCT in Tansania ausgesetzt waren

Kohorte: 82 Frauen hatten für mindestens 1 Woche AZT genommen (Gruppe 1). Im Median betrugen das Gestationsalter bei Beginn der Prophylaxebeginn 29,1 SSW (Wertebereich 28,0-32,2) und die mediane CD4-Zellzahl 390/µl (267-515). 62 Frauen hatten während der Schwangerschaft kein AZT eingenommen (Gruppe 2). Im Median wiesen diese Frauen eine CD4-Zellzahl von 262/µl (194-474) auf. In den soziodemografischen Charakteristika wie Alter, Größe, Gewicht, Bildungsstatus, Familienstand, Entfernung vom Krankenhaus oder Anzahl der Kinder unterschieden sich die Gruppen nicht signifikant.

Zur Geburt wurden 41 Gruppe-1-Kinder untersucht, deren Mütter AZT während der Schwangerschaft für eine mediane Dauer von 57 Tagen (43-71) eingenommen hatten. Diese Kinder wiesen die mediane CD4-Zellzahl von 350/µl (252-490) auf. Die Blutwerte von 37 der Kinder konnten nach Woche 4-6 und von 16 Kindern nach Woche 12-16 analysiert werden. Die Gruppe-2-Kinder wiesen im Median eine CD4-Zellzahl von 262/µl (194-474) auf. Von ihnen wurden 58 zur Geburt, 23 in Woche 4-6 und 12 in Woche 12-14 in die Analyse eingeschlossen. Die Gruppen wiesen in den Basis-Parametern wie Geschlecht, Gewicht und Apgarwerten keine signifikanten Unterschiede auf.

Hämatologische Veränderungen bei den Müttern: Zur Geburt wurde bei Gruppe-1-Müttern eine signifikant niedrigere Erythrozytenzahl (RBC) (3,66/µl vs. 4,04/µl; $p<0,05$) bei höherem mittleren Erythrozytenvolumen (MCV) (87fl vs. 79fl; $p<0,001$) nachgewiesen. Gleichzeitig hatten sie signifikant mehr Thrombozyten (367.000/µl vs. 273.500/µl; $p<0,05$) als die Gruppe-2-

Mütter. In Wochen 4-6 und 12-14 nach Geburt konnten keine hämatologischen Unterschiede mehr festgestellt werden.

Hämatologische Veränderungen bei den Kindern: Zur Geburt zeigten die Gruppe-1-Kinder signifikant niedrigere mediane Werte von HB (13,3g/dl vs. 15,2g/dl; p<0,001), RBC (3,7/µl vs. 4,5/µl; p<0,001) und Granulozyten (5,0/nl vs. 7,3/nl; p=0,042) als die Gruppe-2-Kinder. Zudem wiesen die Gruppe-1-Kinder signifikant höhere MCV-Werte (106fl vs. 100fl; p=0,001) und Erythrozytenverteilungsbreite (RDW) (17,1 vs. 16,2; p=0,008) auf. Nach einem Monat (IQR 31-36 Tage) waren keine signifikanten Unterschiede der HB-, RBC-, und RDW-Werte und nach drei Monaten (IQR 91-93 Tage) waren keine signifikanten Unterschiede der Granulozytenzahlen und der MCV-Werte mehr feststellbar. Insgesamt wurde zur Geburt bei 26% der Kinder, nach einem Monat bei 29% und nach drei Monaten bei 58% der Kinder eine Anämie festgestellt. Bei insgesamt 37% der Kinder wurde zur Geburt eine Granulozytopenie nachgewiesen. Nach einem Monat zeigten noch 19% eine Granulozytopenie. Wie in Tabelle 1 aufgeführt, waren beim Vergleich der Kindergruppen zur Geburt signifikant mehr Kinder der Gruppe 1 von leichter Anämie und leichter Granulozytopenie betroffen. Nach einem Monat wiesen dagegen signifikant mehr Kinder der Gruppe 2 eine leichte Granulozytopenie auf. Zwei Kinder der Gruppe 1 hatten zum Zeitpunkt der Geburt schwere Anämien, die bei einem Kind bis Monat 1 persistierte, während in Gruppe 2 kein Fall von schwerer Anämie auftrat. Insgesamt zeigten zehn Kinder zur Geburt eine schwere Granulozytopenie, die bei vier Kindern bis Monat 1 andauerten. In Monat 3 waren in beiden Gruppen keine schweren Fälle von Anämie oder Granulozytopenie mehr nachweisbar.

Tabelle 1: Häufigkeit von Anämie und Granulozytopenie bei Kindern der Gruppen 1 und 2.

Anämie	**Geburt (n=78)**			**Monat 1 (n=51)**			**Monat 3 (n=24)**		
	Gruppe 1	**Gruppe 2**	**p**	**Gruppe 1**	**Gruppe 2**	**p**	**Gruppe 1**	**Gruppe 2**	**P**
Grad ≥1	15 (46.9%)	5 (10.9%)	**0.001**	11 (35.5%)	4 (20.0%)	0.35	6 (46.2%)	8 (72.7%)	0.24
Grad ≥3	2 (06.3%)	0 (00.0%)	0.17	1 (03.2%)	0 (00.0%)	1	0 (00.0%)	0 (00.0%)	1
Granulo-zytopenie	**Geburt (n=79)**			**Monat 1 (n=53)**			**Monat 3 (n=25)**		
	Gruppe 1	**Gruppe 2**	**p**	**Gruppe 1**	**Gruppe 2**	**p**	**Gruppe 1**	**Gruppe 2**	**P**
Grad ≥1	17 (51.5%)	12 (26.1%)	**0.033**	3 (09.4%)	7 (33.3%)	**0.039**	1 (07.7%)	2 (16.7%)	0.59
Grad ≥3	6 (18.2%)	4 (08.7%)	0.31	2 (06.3%)	2 (09.5%)	1	0 (00.0%)	0 (00.0%)	1

HIV-Transmissionsraten: Zur Bestimmung der in-utero-Transmissionsrate wurde Nabelschnurplasma von 88 Kindern per PCR untersucht. Die Transmissionsrate zur Geburt betrug für Gruppe-1-Kinder 2,4% (Konfidenzintervall (CI) 0,35%-16,0%) und für Gruppe-2-Kinder 8,5% (CI 3,3%-21,1%). Von 63 Kindern wurden Plasmaproben in Woche 6 nach der Geburt untersucht und die Transmissionsrate nach Kaplan-Meier geschätzt. Sie ergab 7,7% für Gruppe 1 (CI 2,5%-22,1%) und 12,5% für Gruppe 2 (CI 5,2%-28,5%).

7 Diskussion

In der vorgestellten Beobachtungsstudie wurde die Umsetzung eines komplexen MTCT-Prophylaxeregimes im ländlichen Tansania evaluiert. Dafür wurden in drei Substudien Medikamentenadhärenz, Resistenzbildungen und hämatotoxische Nebenwirkungen der eingesetzten antiretroviralen Medikamente untersucht.

Die Medikamentenadhärenz war insgesamt besorgniserregend, da nur eine von 120 Müttern mit ihrem Kind ein 95%iges Adhärenzniveau für alle Phasen der Prophylaxeintervention erreichte. Dies ist insbesondere bedenklich, da eine regelmäßige Medikamenteneinnahme eine wichtige Voraussetzung für die viruslastsenkende Wirkung der antiretroviralen Medikamente und die damit verbundene Transmissionsreduktion darstellt.
Fast 30% der Frauen, die sich für eine antiretrovirale Kombinationsprophylaxe entschieden hatten, holten während der Schwangerschaft keine Medikamente im KDH ab. Relativ junges Alter der Mutter und das Fehlen einer einkommensgenerierenden Tätigkeit waren bereits zuvor als Risikofaktoren dafür erkannt worden, nicht mit einer therapeutischen oder prophylaktischen Maßnahme gegen eine HIV-Infektion zu beginnen[11,22]. Darüber hinaus wurde in unserer Studie aufgezeigt, dass auch eine frühe Vorstellung in ANC den Beginn der medikamentösen Prophylaxe während der Schwangerschaft negativ beeinflusst. Eine plausible Erklärung bietet der lange Zeitraum zwischen Aufklärung über die Notwendigkeit der medikamentösen Prophylaxe und tatsächlicher erster Tabletteneinnahme in SSW 28. Um diese zeitliche Lücke zu verkürzen und den PMTCT-Ablauf insgesamt zu vereinfachen, empfiehlt die WHO seit 2010 einen AZT-Prophylaxebeginn bereits ab SSW 14 (Option A)[23]. Mit dem Ziel, die Fälle von Prophylaxeablehnung während der Schwangerschaft zu reduzieren, wurde diese Änderung auch in die aktuellen Tansanischen Leitlinien übernommen[24].
Nur die Hälfte der Frauen, die mit der Einnahme der AZT-Prophylaxe begonnen hatten, erreichte in der vorgeburtlichen Phase ein Adhärenzniveau von mindestens 95%. Adhärenzstudien zu allgemeinen antiretroviralen Therapieprogrammen lassen zwar vermuten, dass theoretisch höhere Adhärenzlevel erreichbar sind[25]. Allerdings sind allgemeine Therapieprogramme nur bedingt mit Interventionen vergleichbar, die ausschließlich schwangere Frauen betreffen. Insofern wären weitere Untersuchungen zur Adhärenz bei komplexen PMTCT-Regimen hilfreich, um die Ergebnisse dieser Studie besser einordnen zu können.
Die Angabe der HIV-Statusoffenbarung gegenüber einer Vertrauensperson wurde als einziger signifikanter Einflussfaktor auf die vorgeburtliche Medikamentenadhärenz identifiziert. Es wird angenommen, dass die Statusoffenbarung emotionalen Stress der Schwangeren reduziert und zu psychologischer und materieller Unterstützung durch das soziale Umfeld beiträgt[26]. Insbesondere in ländlichen Gegenden dürfte dies eine große Rolle für eine regelmäßige Abholung und Ein-

nahme der Medikamente spielen. Über 90% der Studienteilnehmerinnen, die mindestens einmal ins KDH zurückgekehrt waren, gaben an, ihren HIV-Status einer Vertrauensperson mitgeteilt zu haben. Aufgrund der großen Anzahl von *lost to follow-up*-Fällen, die zur Statusoffenbarung nicht befragt werden konnten, ist jedoch anzunehmen, dass die wahre Rate der Statusoffenbarungen deutlich darunter liegt. Gleichzeitig nahmen nur wenige der Studienteilnehmerinnen die im KDH angebotene Möglichkeit einer gemeinsamen HIV-Beratung und -Testung mit dem Partner in Anspruch. Insgesamt gibt der Zusammenhang zwischen Statusoffenbarung und besserer Medikamentenadhärenz Anlass zur Vermutung, dass eine stärkere Einbindung der Partner oder anderer Vertrauenspersonen z.B. in Form von sogenannter *treatment buddies*[27] möglicherweise auch zu einer Reduktion der hohen Anzahl an frühen *lost to follow-up*-Fällen geführt hätte.
Gründe für verpasste Medikamentenabholungen während der Schwangerschaft waren meist persönlicher Art. Zusätzlich bildeten jedoch auch Fehler des Gesundheitspersonals eine relevante Barriere zur regelmäßigen vorgeburtlichen Medikamenteneinnahme. In der intra- und postpartalen Phase der Medikamenteneinnahme wurde die Verantwortung des Personals für die Adhärenz besonders deutlich: Weniger als die Hälfte der aufgenommenen Mütter und nur ein Fünftel ihrer Kinder erreichten 95% Adhärenz während des stationären Entbindungsaufenthaltes. Außerdem erhielten nur etwa 80% der Mütter und ihrer Kinder die richtige Dosierung der ambulant einzunehmenden Medikamente. Die fehlerhafte Verabreichung der Medikamente ist zumindest zu einem gewissen Teil der hohen Arbeitsbelastung des Gesundheitspersonals geschuldet. Hinzu kommt oft mangelndes PMTCT-Wissen des Personals, da zum Zeitpunkt der Datenerhebung häufige Personalrotationen zwischen den verschiedenen Stationen im KDH üblich waren. Diese erschweren ein intensives Training von speziellen PMTCT-Kräften[28]. Daher wäre eine deutliche Vereinfachung der PMTCT-Leitlinien wünschenswert. Unter anderem aus diesem Grund favorisiert die WHO seit 2012 den Beginn einer lebenslangen ART für alle Schwangeren Frauen[29], die auch in Tansania in Erwägung gezogen werden sollte. Die Reduktion krankenhausinterner Rotationssysteme und eine sorgfältige PMTCT-Schulung des gesamten Gesundheitspersonals könnten zusätzlich dazu beitragen, dass sowohl stationär als auch ambulant höhere Adhärenzniveaus erreicht werden und dadurch die Voraussetzung für die erwünschte Transmissionsreduktion sichergestellt wird.

Plasmaproben von 50 Studienteilnehmerinnen wurden in der Resistenzanalyse untersucht, da die Frauen sich den Einschlusskriterien für Substudie 2 entsprechend an mindestens fünf Terminen im KDH vorgestellt hatten. Bei 40% dieser Frauen wurden minoritäre HIV-Varianten gefunden, die Resistenzen gegen ein oder mehrere ARVs aufwiesen. Dies ist eine bedenklich hohe Resistenzrate, da die häufigen Krankenhausbesuche eine relativ gute Adhärenz von Seiten der Schwangeren annehmen lässt. In Fällen, in denen die Frauen sich weniger regelmäßigen im

KDH vorstellten und daher in dieser Studie nicht berücksichtigt werden konnten, sind noch höhere Resistenzraten zu befürchten. Bei 22% der Frauen konnten AZT-resistente HI-Viren nachgewiesen werden, die jedoch zum größten Teil als minoritäre Virusvarianten vorlagen. In 8% der Fälle war die K70R-Mutation als frühe Mutation bereits zur Geburt nachweisbar, während T215Y und T215F als späte Mutationen erst im Verlauf auftraten. Die K70R-Mutation ist transient, T215Y und T215F können dagegen bis mehrere Monate nach Absetzen von AZT persistieren[30]. Dies ist besonders relevant, wenn in diesem Zeitraum eine erneute AZT-haltige Prophylaxe oder Therapie begonnen wird. Gerade in Subsahara-Afrika, wo Frauen meist mehrere Kinder in relativ kurzen zeitlichen Abständen gebären, können früher entstandene AZT-Resistenzen bei wiederholtem Einsatz von AZT in einer folgenden Schwangerschaft erneut selektiert werden. Auf diese Weise ist eine Anhäufung von AZT-resistenten Viren möglich, die die Wirksamkeit von AZT einschränken. Da eine hohe Viruslast und eine niedrige CD4-Zellzahl mit dem Auftreten von AZT-Resistenzen assoziiert sind[31], ist es sinnvoll, bereits bei einer CD4-Zellzahl von <350/µl mit einer ART zu beginnen, wie es auch in den WHO-Leitlinien von 2010 favorisiert und in Tansania bereits umgesetzt wird[23,24]. Die von der WHO empfohlenen Möglichkeiten, bereits während der Schwangerschaft mit einer Dreifachmedikamentenkombination (Option B) oder, wie seit 2012 angedacht, einer lebenslangen ART für alle HIV-postitiven Schwangeren zu beginnen (Option B+)[29], werden in Tansania hingegen noch nicht genutzt. Allerdings wurden AZT-selektierte HI-Varianten zum Zeitpunkt der Geburt signifikant häufiger bei Frauen nachgewiesen, die während der Schwangerschaft für mehr als 10 Wochen AZT eingenommen hatten. Auch andere Studien konnten die Einnahmedauer einer AZT-Monotherapie mit einer vermehrten Entstehung von Resistenzen in Verbindung bringen[31]. Es erscheint daher äußerst sinnvoll, auch in Tansania, wo aktuell bereits in SSW 14 mit einer Medikamenteneinnahme begonnen wird, einem vermehrten Auftreten von Resistenzen durch eine antiretrovirale Kombinationsprophylaxe oder -therapie vorzubeugen.

NVP und andere NNRTIs stellen gerade in ressourcenschwachen Regionen eine der wichtigsten Säulen der ART dar. Daher geben NVP-selektierte Resistenzmutationen, die zusätzlich Kreuzresistenzen gegen andere NNRTIs hervorrufen können, grundsätzlich Anlass zu Sorge. In unserer Studie wurden NVP-resistente HIV-Varianten bei 18% der Frauen gefunden, eine drastische Reduktion im Vergleich zu einer Resistenzrate von 87% nach NVP-ED[4].

Die 3TC-Resistenz M184 konnte bei HI-Viren in nur 8% der Fälle und in äußerst geringen Proportionen von <1% nachgewiesen werden. Die klinische und virologische Relevanz solch geringer Proportionen der M184V-Mutation, die nach Absetzen von 3TC nur kurzzeitig erhalten bleibt, ist derzeit unklar.

Bei drei der 50 Frauen wurden Virusvarianten mit Resistenzen gegen mehr als ein ARV gefunden. Der Hauptrisikofaktor für die ARV-Resistenzentwicklung ist eine suboptimale Adhärenz.

Die am stärksten von resistenten HI-Viren betroffene Frau hatte die postpartale AZT/3TC-Prophylaxe nicht eingenommen, was höchstwahrscheinlich die Entstehung der Multiresistenz deutlich begünstigt hat. An diesem Beispiel werden mögliche negative Konsequenzen des komplexen Prophylaxeregimes besonders deutlich.
Auch bei drei der sieben HIV-infizierten Kinder wurden medikamentenresistente Virusvarianten gefunden. Bei zwei von drei Kindern, die NVP-selektierte HI-Virusvarianten aufwiesen, traten die RT-Mutationen erstmals nach Infektion der Kinder auf: Im mütterlichen Blut konnten keine entsprechenden resistenten HI-Viren nachgewiesen werden. Bei einem Kind, welches nach der Geburt selber kein NVP erhalten hatte, wurden in Woche 4-6 NVP-resistente Virusvarianten in hohen Proportionen nachgewiesen. Dies ist möglich, da das von der Mutter zur Geburt eingenommene NVP über die Plazenta rasch in den kindlichen Blutkreislauf gelangt und dort hohe Konzentrationen erreicht[32], die für eine Resistenzbildung ausreichend sind.

Die in der Hämatotoxizitätsanalyse festgestellten signifikanten Blutbildveränderungen der AZT-exponierten Gruppe-1-Mütter zur Geburt waren bereits nach wenigen Wochen nicht mehr nachweisbar. Die zur Geburt im Vergleich zu Gruppe 2 signifikanten Blutbildveränderungen der Gruppe-1-Kinder, wie niedrigere Granulozytenzahlen und niedrigere HB-Werte stehen im Einklang mit früheren Studienergebnissen, in denen bereits Anämie[15] und Granulozytopenie[16] bei in-utero AZT-exponierten Kindern beschrieben wurden. Auch dass die HB-Unterschiede der Gruppen nach einem Monat nicht mehr signifikant waren, deckt sich mit diesen Ergebnissen[15]. Das Phänomen ist sehr wahrscheinlich auf die 4-wöchige postpartale AZT-Einnahmedauer der Gruppe-2-Kinder und die daraus resultierende anhaltende HB-Abnahme zurückzuführen. Gruppe-1-Kinder bekamen entsprechend der Leitlinien postpartal für nur eine Woche AZT. Ihre Blutwerte konnten sich dementsprechend schneller wieder erholen. Auch das nach einem Monat signifikant häufigere Auftreten von Granulozytopenie bei Kindern der Gruppe 2 lässt sich mit der längeren Einnahmedauer von AZT erklären. Mit dem Alter von einem Monat hatten 9% der Gruppe-2-Kinder eine schwere Granulozytopenie (≥Grad 3) entwickelt, die höchstwahrscheinlich auf die in-utero und postpartale AZT-Exposition zurückzuführen ist.
Die Kontrolle der Blutbilder fand hauptsächlich im Rahmen der Studie statt und wird routinemäßig im KDH wegen der finanziellen und logistischen Anforderungen nur selten durchgeführt. Allerdings stellen fehlende Laborkontrollen in Fällen schwerer hämatotoxischer Nebenwirkungen für die Betroffenen eine große Gefahr dar. Eine mangelhafte Überwachung toxischer Medikamentennebenwirkungen sollte daher bei der Umsetzung komplexer PMTCT-Regime in Gegenden mit limitierten Ressourcen nicht unberücksichtigt bleiben. Vielmehr wäre die Einführung einer routinemäßigen Überwachung der Nebenwirkungen für alle medikamentösen PMTCT-Interventionen erstrebenswert, um eine ausreichende Interventionssicherheit zu gewährleisten.

Die insgesamt schwache Adhärenz wirft die Frage nach der tatsächlichen Transmissionsreduktion durch die antiretrovirale Kombinationsprophylaxe auf. Die in Substudien 2 und 3 berechneten Transmissionsraten sind jedoch wegen der geringen Stichprobengrößen der betrachteten Subkohorten nur eingeschränkt aussagekräftig. Die in Substudie 2 berechnete HIV-Übertragungsrate nach Woche 6 erreicht trotz Einnahme der medikamentösen Kombinationsprophylaxe 14,3%. Eine derartig hohe Rate wäre eher nach der alleinigen Prophylaxe mit NVP-ED zu erwarten gewesen[3]. Eine Vergleichsgruppe ohne AZT-Einnahme lag nicht vor. In Substudie 3 wurden HIV-Übertragungswahrscheinlichkeit für Woche 6 per Kaplan-Meier-Schätzer bestimmt. Sie betrug 7,7% in Gruppe 1 (mit AZT), ohne sich signifikant von den für Gruppe 2 (ohne AZT) berechneten 12,5% zu unterscheiden. Dennoch steht die Größenordnung dieses Ergebnisses im Einklang mit den Ergebnissen einer früheren Studie, in der nach vierwöchiger AZT-Einnahme in der Schwangerschaft und NVP-ED zur Geburt eine Transmissionswahrscheinlichkeit von 6,5% berechnet wurde[33].

Zusammenfassend stellte die Adhärenz zu einem komplexen antiretroviralen MTCT-Prophylaxeregime in einer peripheren Region Tansanias eine große Herausforderung dar, sowohl für die betroffenen Mütter, als auch für das Gesundheitspersonal. Die tatsächliche Reduktion der HIV-Übertragungsraten ist dadurch in Frage gestellt. Gleichzeitig fand die Entstehung von Resistenzen gegen eingesetzte Medikamente häufig, wenn auch hauptsächlich als minoritäre HI-Virusvarianten und in geringeren Proportionen als bei einmaldosierten Regimes statt. Zur Steigerung der Medikamentenadhärenz und Vorbeugung von Resistenzbildungen wären zusätzlich zu den bisherigen Leitlinienanpassungen an die WHO-Empfehlungen von 2010 eine stärkere Unterstützung der betroffenen Frauen durch enge Vertrauenspersonen und eine nachhaltigere Schulung des Personals hilfreich. Die nach Medikamenteneinnahme beobachteten Blutbildveränderungen der Mütter waren hingegen meist milder Natur. Da gerade in ressourcenschwachen Regionen oft auf routinemäßige Blutbildkontrollen verzichtet wird, sollten die bei einigen wenigen Kindern aufgetretenen Fälle schwerer Hämatotoxizität jedoch nicht vernachlässigt werden. Ganz im Gegenteil erscheint eine routinemäßige Überwachung der Blutwerte als fester Bestandteil einer komplexen medikamentösen PMTCT-Intervention angebracht.
Um das von der Weltgemeinschaft hoch gesteckte Ziel zu erreichen, die perinatale HIV-Infektion bis 2015 zu eliminieren[34], bedarf es weiterhin größter Anstrengung, PMTCT-Regime insbesondere für ihren Einsatz in ländlichen Gegenden zu optimieren. Dafür wäre in Tansania die Ausweitung der ARV-Kombinationsprophylaxe auf den Beginn einer lebenslangen ART für alle Schwangeren nicht zuletzt hinsichtlich der anzunehmenden positiven Auswirkungen auf Adhärenz, Resistenzbildungen und Wirksamkeit äußerst wünschenswert.

8 Literaturverzeichnis

1. Joint United Nations Programme on HIV/AIDS (UNAIDS). Global report: UNAIDS report on the global AIDS epidemic 2010. Geneva: UNAIDS/WHO; 2010.

2. De Cock KM, Fowler MG, Mercier E, et al. Prevention of mother-to-child HIV transmission in resource-poor countries: translating research into policy and practice. Jama 2000;283:1175-82.

3. Guay LA, Musoke P, Fleming T, et al. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomised trial. Lancet 1999;354(9181):795-802.

4. Arrive E, Newell ML, Ekouevi DK, et al. Prevalence of resistance to nevirapine in mothers and children after single-dose exposure to prevent vertical transmission of HIV-1: a meta-analysis. Int J Epidemiol 2007;36(5):1009-21.

5. World Health Organization (WHO): HIV/AIDS Programme. Antiretroviral drugs for treating pregnant women and preventing HIV infections in infants: towards universal access, recommendations for a public health approach. Geneva: WHO Press; 2006.

6. United Nations Development Programme (UNDP). Human development report 2011, sustainability and equity: a better future for all. New York: Palgrave Macmillan; 2011.

7. The United Republic of Tanzania: Ministry of Health and Social Welfare. Prevention of mother-to-child transmission (PMTCT): national guidelines, December 2007. Dar Es Salaam: Ministry of Health and Social Welfare; 2007.

8. Gross R, Bilker WB, Friedman HM, Strom BL. Effect of adherence to newly initiated antiretroviral therapy on plasma viral load. Aids 2001;15(16):2109-17.

9. Garcia PM, Kalish LA, Pitt J, et al. Maternal levels of plasma human immunodeficiency virus type 1 RNA and the risk of perinatal transmission. N Engl J Med 1999;341(6):394-402.

10. Smith RJ. Adherence to antiretroviral HIV drugs: how many doses can you miss before resistance emerges? Proc Biol Sci 2006;273(1586):617-24.

11. Karcher H, Kunz A, Poggensee G, Mbezi P, Mugenyi K, Harms G. Outcome of different nevirapine administration strategies in preventing mother-to-child transmission (PMTCT) programs in Tanzania and Uganda. MedGenMed 2006;8(2):12.

12. Nolan M, Fowler MG, Mofenson LM. Antiretroviral prophylaxis of perinatal HIV-1 transmission and the potential impact of antiretroviral resistance. J Acquir Immune Defic Syndr 2002;30(2):216-29.

13. Johnson JA, Li JF, Wei X, et al. Minority HIV-1 drug resistance mutations are present in antiretroviral treatment-naive populations and associate with reduced treatment efficacy. PLoS Med 2008;5(7):e158.

14. MacLeod IJ, Rowley CF, Thior I, et al. Minor resistant variants in nevirapine-exposed infants may predict virologic failure on nevirapine-containing ART. J Clin Virol 2010;48(3):162-7.

15. Connor EM, Sperling RS, Gelber R, et al. Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. N Engl J Med 1994;331(18):1173-80.

16. Le Chenadec J, Mayaux MJ, Guihenneuc-Jouyaux C, Blanche S. Perinatal antiretroviral treatment and hematopoiesis in HIV-uninfected infants. Aids 2003;17(14):2053-61.

17. Arrive E, Dabis F. Prophylactic antiretroviral regimens for prevention of mother-to-child transmission of HIV in resource-limited settings. Curr Opin HIV AIDS 2008;3(2):161-5.

18. The world factbook, Tanzania. Central Intelligence Agency (CIA), 2012. (Accessed November 18, 2012, at https://www.cia.gov/library/publications/the-world-factbook/geos/tz.html.)

19. Harms G, Kunz A, Theuring S. German HIV practice collection: prevention of mother-to-child transmission of HIV in Kenya, Tanzania and Uganda. Eschborn: The German HIV Peer Review Group; 2007.

20. World Health Organization (WHO). Chronic HIV care with ARV therapy and prevention. Geneva: WHO Press; 2007.

21. Johnson VA, Brun-Vezinet F, Clotet B, et al. Update of the drug resistance mutations in HIV-1: December 2010. Top HIV Med 2010;18(5):156-63.

22. Byakika-Tusiime J, Oyugi JH, Tumwikirize WA, Katabira ET, Mugyenyi PN, Bangsberg DR. Adherence to HIV antiretroviral therapy in HIV+ Ugandan patients purchasing therapy. Int J STD AIDS 2005;16(1):38-41.

23. World Health Organization (WHO). Antiretroviral drugs for treating pregnant women and preventing HIV infection in infants: recommendations for a public health approach, 2010 version. Geneva, Switzerland: WHO Press; 2010.

24. The United Republik of Tanzania: Ministry of Health and Social Welfare. National guidelines for comprehensive care of PMTCT of HIV services. 3rd ed. Dar Es Salaam: Ministry of Health and Social Welfare; 2012.

25. Mills EJ, Nachega JB, Buchan I, et al. Adherence to antiretroviral therapy in sub-Saharan Africa and North America: a meta-analysis. Jama 2006;296(6):679-90.

26. Norman A, Chopra M, Kadiyala S. Factors related to HIV disclosure in 2 South African communities. Am J Public Health 2007;97(10):1775-81.

27. Wouters E, Van Damme W, van Rensburg D, Meulemans H. Impact of baseline health and community support on antiretroviral treatment outcomes in HIV patients in South Africa. Aids 2008;22(18):2545-8.

28. Nuwagaba-Biribonwoha H, Mayon-White RT, Okong P, Carpenter LM. Challenges faced by health workers in implementing the prevention of mother-to-child HIV transmission (PMTCT) programme in Uganda. J Public Health (Oxf) 2007;29(3):269-74.

29. World Health Organization (WHO). Programmatic update: use of antiretroviral drugs for treating pregnant women and preventing HIV infection in infants, executive summary. Geneva: WHO Press; 2012.

30. Albert J, Wahlberg J, Lundeberg J, et al. Persistence of azidothymidine-resistant human immunodeficiency virus type 1 RNA genotypes in posttreatment sera. J Virol 1992;66:5627-30.

31. Land S, McGavin C, Lucas R, Birch C. Incidence of zidovudine-resistant human immunodeficiency virus isolated from patients before, during, and after therapy. J Infect Dis 1992;166(5):1139-42.

32. Kunz A, Frank M, Mugenyi K, et al. Persistence of nevirapine in breast milk and plasma of mothers and their children after single-dose administration. J Antimicrob Chemother 2009;63(1):170-7.

33. Dabis F, Bequet L, Ekouevi DK, et al. Field efficacy of zidovudine, lamivudine and single-dose nevirapine to prevent peripartum HIV transmission. Aids 2005;19(3):309-18.

34. Countdown to zero: global plan for the elimination of new HIV infections among children by 2015 and keeping their mothers alive, 2011-2015. UNAIDS/WHO, 2011. (Accessed January 7, 2013, at http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2011/20110609_JC2137_Global-Plan-Elimination-HIV-Children_en.pdf.)

Eidesstattliche Versicherung

Ich, Inga Lau, geb. Kirsten, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „Vertikale HIV-Transmission im ländlichen Tansania: Herausforderungen bei der Umsetzung eines komplexen antiretroviralen Prophylaxeregimes“ selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung (siehe „Uniform Requirements for Manusripts (URM)“ des ICMJE – *www.**icmje**.org*) kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) entsprechen den URM (s.o.) und werden von mir verantwortet.

Meine Anteile an den ausgewählten Publikationen entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit der Betreuerin angegeben sind. Sämtliche Publikationen, die aus dieser Dissertation hervorgegangen sind und bei denen ich Autor bin, entsprechen den URM (s.o.) und werden von mir verantwortet.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst.

_____

Datum Unterschrift

Anteilserklärung an den erfolgten Publikationen

Inga Lau hatte folgenden Anteil an den dieser Arbeit zugrunde liegenden Publikationen:

Publikation 1:

Kirsten I, Sewangi J, Kunz A, Dugange F, Ziske J, Jordan-Hader B, Harms G, Theuring S. *Adherence to Combination Prophylaxis for Prevention of Mother-to-Child-Transmission of HIV in Tanzania.* PLoS ONE 2011;6(6):e21020.

Beitrag im Einzelnen:
- Mitarbeit in der Planung zur Umsetzung der Studie
- Mitarbeit bei der Erstellung der Fragebögen
- Supervision der Studiendurchführung in Tansania: Rekrutierung der Studienteilnehmerinnen; Probenabnahme und Probenverarbeitung; Datenerhebung und Datendokumentation
- Dateneingabe und Datenaufbereitung; statistische Analysen
- Erstellung der Erstversion des Manuskripts und Vorbereitung der Publikation

Publikation 2:

Hauser A, Sewangi J, Mbezi P, Dugange F, **Lau I**, Ziske J, Theuring S, Kuecherer C, Harms G, Kunz A. *Emergence of Minor Drug-Resistant HIV-1 Variants after Triple Antiretroviral Prophylaxis for Prevention of Vertical HIV-1 Transmission.* PLoS ONE 2012;7(2):e32055.

Beitrag im Einzelnen:
- Mitarbeit bei der Erstellung der Fragebögen
- Supervision der Studiendurchführung in Tansania: Rekrutierung der Studienteilnehmerinnen; Probenabnahme und Probenverarbeitung; Datenerhebung und Datendokumentation

Publikation 3:

Ziske J, Kunz A, Sewangi J, **Lau I**, Dugange F, Hauser A, Kirschner W, Harms G, Theuring S. *Hematological changes in women and infants exposed to AZT-containing regimen for prevention of mother-to-child-transmission of HIV in Tanzania.* PLoS ONE 2013;8(2):e55633.

Beitrag im Einzelnen:
- Mitarbeit in der Planung der Umsetzung der Studie
- Mitarbeit bei der Erstellung der Fragebögen
- Mitarbeit in der Studiendurchführung in Tansania: Rekrutierung der Studienteilnehmerinnen; Datenerhebung und Datendokumentation; Probenabnahme und Probenverarbeitung;
- Mitwirken bei der Manuskripterstellung

Unterschrift, Datum und Stempel der betreuenden Hochschullehrerin

_____

Unterschrift der Doktorandin

_____

Druckexemplar Publikation 1

Kirsten I, Sewangi J, Kunz A, Dugange F, Ziske J, Jordan-Harder B, Harms G, Theuring S

Adherence to Combination Prophylaxis for Prevention of Mother-to-Child-Transmission of HIV in Tanzania

PLoS ONE 2011;6(6):e21020

doi:10.1371/journal.pone.0021020

OPEN ACCESS Freely available online

PLoS one

Adherence to Combination Prophylaxis for Prevention of Mother-to-Child-Transmission of HIV in Tanzania

Inga Kirsten[1], Julius Sewangi[2], Andrea Kunz[1], Festo Dugange[3], Judith Ziske[1], Brigitte Jordan-Harder[1], Gundel Harms[1], Stefanie Theuring[1]*

1 Institute of Tropical Medicine and International Health, Charité-Universitätsmedizin Berlin, Berlin, Germany, **2** Regional AIDS Control Program Mbeya Region, Ministry of Health and Social Welfare, Dar es Salaam, Tanzania, **3** Kyela District Hospital, Ministry of Health and Social Welfare, Dar es Salaam, Tanzania

Abstract

Background: Since 2008, Tanzanian guidelines for prevention of mother-to-child-transmission of HIV (PMTCT) recommend combination regimen for mother and infant starting in gestational week 28. Combination prophylaxis is assumed to be more effective and less prone to resistance formation compared to single-drug interventions, but the required continuous collection and intake of drugs might pose a challenge on adherence especially in peripheral resource-limited settings. This study aimed at analyzing adherence to combination prophylaxis under field conditions in a rural health facility in Kyela, Tanzania.

Methods and Findings: A cohort of 122 pregnant women willing to start combination prophylaxis in Kyela District Hospital was enrolled in an observational study. Risk factors for decline of prophylaxis were determined, and adherence levels before, during and after delivery were calculated. In multivariate analysis, identified risk factors for declining pre-delivery prophylaxis included maternal age below 24 years, no income-generating activity, and enrolment before 24.5 gestational weeks, with odds ratios of 5.8 (P = 0.002), 4.4 (P = 0.015) and 7.8 (P = 0.001), respectively. Women who stated to have disclosed their HIV status were significantly more adherent in the pre-delivery period than women who did not (P = 0.004). In the intra- and postpartum period, rather low drug adherence rates during hospitalization indicated unsatisfactory staff performance. Only ten mother-child pairs were at least 80% adherent during all intervention phases; one single mother-child pair met a 95% adherence threshold.

Conclusions: Achieving adherence to combination prophylaxis has shown to be challenging in this rural study setting. Our findings underline the need for additional supervision for PMTCT staff as well as for clients, especially by encouraging them to seek social support through status disclosure. Prophylaxis uptake might be improved by preponing drug intake to an earlier gestational age. Limited structural conditions of a healthcare setting should be taken into serious account when implementing PMTCT combination prophylaxis.

Citation: Kirsten I, Sewangi J, Kunz A, Dugange F, Ziske J, et al. (2011) Adherence to Combination Prophylaxis for Prevention of Mother-to-Child-Transmission of HIV in Tanzania. PLoS ONE 6(6): e21020. doi:10.1371/journal.pone.0021020

Editor: Landon Myer, University of Cape Town, South Africa

Received December 6, 2010; **Accepted** May 18, 2011; **Published** June 10, 2011

Funding: The study was supported by the German Ministry of Economic Cooperation through the GTZ Sector Project "Strengthening the German Contribution to the Global AIDS Response". The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: stefanie.theuring@charite.de

Introduction

Worldwide, more than two million children younger than 15 years are HIV positive, with 90% of those living in Subsaharan Africa. 370,000 children were newly infected in 2009 [1], mostly by mother-to-child-transmission (MTCT) during pregnancy, during delivery or after delivery via breastfeeding. Without medical intervention, transmission rates range between 25% and 48% in resource-limited settings [2].

For prevention of mother-to-child transmission of HIV (PMTCT), administration of a single dose of the non-nucleoside reverse transcriptase inhibitor (NNRTI) nevirapine (NVP) to both mother and newborn has shown to reduce the transmission risk by over 40% [3,4]. Single-dosed NVP (sdNVP) is cheap and easy to administer [4]. However, it has been shown that transmission reduction is considerably more effective when combining sdNVP with two nucleoside reverse transcriptase inhibitors (NRTIs), such as zidovudine (AZT) and lamivudine (3TC) [5,6]. At the same time, sdNVP is prone to resistance formation and might impede subsequent treatment involving NVP or other NNRTIs [7,8], while combining NVP with NRTIs has shown to reduce the emergence of NNRTI-resistant mutations [8,9]. Since 2006, the World Health Organization (WHO) therefore recommends a triple combination prophylaxis regimen consisting of two NRTIs (antenatal AZT, intra/postpartum AZT+3TC) and one NNRTI (intrapartum sdNVP) as the standard PMTCT regimen wherever this is feasible [10].

The United Republic of Tanzania is one of the poorest and least developed countries in the world [11], and has an overall HIV prevalence of about 6% [12]. HIV prevalence in pregnant women is estimated at 10–16% [13]. In 2008, the Tanzanian Ministry of Health followed the 2006 WHO guidelines for PMTCT and changed its national standard recommendation from sdNVP to combination prophylaxis. The recommended regimen includes

AZT 300 mg twice a day starting in week 28 of pregnancy or as soon as possible thereafter. With the onset of labor, women should take sdNVP, AZT 300 mg every 3 hours, and 3TC 150 mg every 12 hours until delivery. After delivery, a postpartum tail of AZT 300 mg and 3TC 150 mg twice a day should be continued for seven days. All newborns of HIV-positive mothers should receive 2 mg/kg sdNVP within 72 hours and a postpartum tail of 4 mg/kg AZT twice a day for seven days if the mother took AZT during pregnancy for four weeks or longer. Otherwise, the infant postpartum tail should last for four weeks. In both the Tanzanian and WHO recommendations, sdNVP only remains the minimum prophylactic standard for PMTCT if more complex interventions are not feasible [14]. Notably, while Tanzanian guidelines at time of study conduction were based on 2006 WHO recommendations, those were again revised in 2010. WHO now recommends start of AZT intake from gestational week 14 onwards, suggesting that an omission of sdNVP can be considered if AZT was taken for more than four weeks before delivery [15].

Optimal drug adherence is crucial for drug effectiveness: on the one hand, to sufficiently suppress the maternal viral load [16], which in turn is one of the most important risk factors for MTCT [17]. On the other hand, maladherence to antiretroviral drugs potentially promotes the emergence of resistant viral strains which may lead to failure of subsequent treatment [18].

Studies regarding adherence to combination prophylaxis for PMTCT in rural, structurally limited settings are largely lacking up to today. However, previous research on the acceptance of simpler PMTCT regimens showed that in peripheral settings of Tanzania, Uganda and Zambia, sdNVP was accepted by only 20–60% of HIV-positive women seeking antenatal care (ANC) [4,19]. This allows for hypothesizing that more complex regimens might inherit an even greater risk of suboptimal adherence in rural areas, and that combination PMTCT prophylaxis might not bring the expected benefit compared to sdNVP in terms of reduced transmission rates and emerging resistance. The aim of this study has been to gain insight into adherence to combination prophylaxis in one exemplary district hospital in rural Tanzania, and to identify possible challenges of adherence when implementing this regimen.

Methods

The study was undertaken at Kyela District Hospital (KDH), which is a rural health facility in Mbeya Region, Tanzania, and has been providing PMTCT services supported by the German Agency for Technical Co-operation since 2001. There are approximately 130 pregnant women who approach KDH as new ANC clients every month. While adult HIV prevalence in Mbeya Region is found at around 8% [20], HIV prevalence reached 18% among pregnant women in KDH in 2007 (unpublished data). Combination prophylaxis was introduced as the standard PMTCT regimen in KDH in March 2008. KDH was the only facility offering the extended regimen in the vicinity at time of study conduction, while other closeby facilities offered sdNVP only. Our study observed the routine ANC-embedded PMTCT procedure in KDH between October 2008 and September 2009.

The procedure comprised provider-initiated voluntary HIV-counseling and testing of all pregnant women approaching ANC services. Couple testing was encouraged. In HIV-positive women, CD4-cell count was performed to assess indication for antiretroviral therapy (ART). Those with a CD4-cell count$<$200 cells/µl at stage 1 or 2 of the WHO clinical staging [21], CD4-count$<$350 cells/µl at stage 3 and women at stage 4 regardless of CD4-count were referred to ART. These women were automatically ineligible for study participation. Women not indicated for ART were offered combination prophylaxis according to the new Tanzanian guidelines if not showing contraindications like severe anemia (hemoglobin$<$7.5 g/dl) or granulocytopenia (granulocytes$<$ $0.75*10^9$/l) [14]. Consenting to take part in the prophylaxis intervention was considered as PMTCT enrollment. The PMTCT intervention required women to start AZT intake in gestational week 28, or as soon as possible thereafter. According to Tanzanian PMTCT guidelines, women were scheduled to collect their AZT supply weekly at KDH for the first month of prophylaxis and monthly thereafter. Drug collection required an ANC visit and a subsequent visit of KDH pharmacy.

All women were strongly advised to deliver in KDH, or to return to KDH within 72 hours postpartum if delivery elsewhere was unavoidable. To ensure sdNVP intake at the onset of labor regardless of the place of delivery, women having reached gestational week 28 were handed out maternal sdNVP for possible self-administration. For deliveries in KDH, maternity staff dispensed drugs to mothers and newborns during hospitalization. At discharge, mothers received AZT and 3TC tablets and infant AZT syrup for the remaining postpartum tail to take home. In the case of delivery elsewhere, postnatal infant sdNVP and maternal and infant postpartum drugs to take home were dispensed upon women's return within 72 hours. Women who had not started prophylaxis during pregnancy, but came to KDH for or after delivery, were equally offered intra/postpartum prophylaxis.

Between October 2008 and July 2009, PMTCT-enrolling women were systematically included in the observational study if they fulfilled the eligibility criteria of written informed consent, $>$18 years of age and no signs of psychological disorder. Confidentiality of all obtained data was ensured. Study participation did not involve additional hospital visits beyond those necessary for the PMTCT intervention.

Data collection

Standardized questionnaires for the stages of ANC, delivery and maternity were developed by the authors and modified during a pretesting period of one week before start of the study.

Data collection included an initial assessment of sociodemographic, socioeconomic and clinical parameters at the point of consenting to participate in the study. Observations were then divided into a pre-delivery and an intra/postpartum period, giving consideration to respective different implications for adherence, i.e. client-driven drug collection during pregnancy and provider-driven intra/postpartum drug dispensation. All stages of data collection were supported by a trained study nurse, ensuring the routine workflow would not be disturbed.

For pre-delivery data collection, study participants were interviewed during their recurrent ANC visits until delivery. The study nurse conducted interviews in Swahili or the local language, and took down answers in English. Pre-delivery questionnaires focused on aspects like drug adherence, status of HIV-disclosure, and reasons for failed drug collection in case of one or more missed drug collection episodes. General acceptance of prophylaxis among study participants was defined as having collected AZT at least once in the pre-delivery period. Those who never collected AZT during pregnancy were considered to be declining prophylaxis. The definition of acceptance and decline was limited to the pre-delivery period because it was assumed that drug receipt during intra/postpartum hospitalization would not to the same extent reflect an active acceptance process.

Intra/postpartum data collection included all study participants who returned to KDH for delivery or within 72 hours thereafter.

Those who had declined pre-delivery prophylaxis, but returned to KDH intra/postpartumly were equally incorporated into this part of observation. Maternity ward nurses, supported by the study nurse, were trained to fill in questionnaires listing all different drugs dispensed in the intra/postpartum period for each woman and newborn, including postpartum tail drugs handed out to take home. Intrapartum AZT and 3TC administration was only observed in KDH deliveries. Study participants who had delivered outside of KDH reported self-administration of sdNVP to staff at their postpartum return; their questionnaires were then continued with information on infant sdNVP intake and dispensed take-home drugs.

Study participants who did not return to KDH for delivery or within 72 hours postpartum were defined as lost to follow-up.

Definition and assessment of adherence

As customary for PMTCT services, drug intake was not directly observed in most parts of the intervention. Accordingly, adherence measures assessed drug collection and dispensation rather than actual ingestion, except during intra/postpartum hopitalisation. Overall adherence was defined to consist of women's drug collection during pregnancy (pre-delivery adherence), and drug dispensation by staff during/after delivery including the postpartum take-home tail (intra/postpartum adherence).

Pre-delivery adherence was measured by women's medication possession ratio (MPR) [22], which was generated from the number of collected AZT doses divided by the targeted number of doses between start of prophylaxis and delivery. Thus, collecting all drugs as scheduled yielded an MPR of 100%, defined as full adherence until delivery. In case of an unknown date of delivery, an estimated delivery date was used to calculate the MPR.

Assessing intra/postpartum adherence was based on staff-listed dispensation of respective drugs. Adherence to sdNVP was measured in absolute intake numbers for mothers and newborns as reported by nurses, or reported by women in case of maternal self-administration. For maternal intrapartum AZT and 3TC, adherence rates were assessed by dividing the hours covered through dispensed drugs by the total hours of hospitalization until delivery. Postpartum tail adherence comprised two parts: first, the observed dispensation of AZT and 3TC to mothers and AZT to newborns before hospital discharge, measured by hours of hospitalization covered with drugs, and second, dispensation of take-home doses for mother and infant after hospital discharge, measured in absolute numbers of women and infants supplied with the correct amount of drugs to take home. Outcome measures of the different observed stages are summarized in Table 1.

Statistical methods

Data analysis was performed with a standard statistical software package. For dichotomization of metric parameters like age and gestational age at enrollment, cut-off-thresholds were defined by using JRip, which is part of the open source machine learning software WEKA 3 [23] and identifies thresholds with the most discriminative power regarding the dependent variable. We set a CD4-count cut-off level at $\leq$350 cells/µl due to the relevance of this value in international guidelines [15]. For the remaining interval variables, the median was used as cut-off threshold. Pearson's chi square test and Fisher's exact test were used to compare categorical data. To evaluate socio-demographic variables and their influence on declining adherence, univariate odds ratios were calculated. Variables that showed to be significant in the univariate analysis ($P<0.05$) were included into a multivariate analysis using a forward stepwise logistic regression to calculate adjusted odds ratios. For analysis of adherence levels, adherence cut-off points of $\geq$95% and $\geq$80% were used for comparability with other studies [24]. The influence of sociodemographic variables on adherence values was tested using the Mann-Whitney U-test.

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki. It was approved by the Tanzanian National Institute for Medical Research, by the Ethical Committee Mbeya, and by the Ethical Committee of Charité-Universitätsmedizin Berlin, Germany. Written informed consent in Swahili was obtained from all participants. Confidentiality was maintained throughout the study.

Results

In total, 1395 women were counseled and HIV-tested in ANC of KDH during the study period. Among 202 women identified HIV-positive (14.5% of all tested), 72 (35.6%) did not meet the eligibility criteria of the study, mostly because of an ART indication. From 130 eligible women, 5 moved away and were therefore referred to another health facility, 2 had an abortion before starting prophylaxis, and 1 withdrew her informed consent.

For the remaining cohort of 122 study participants, the following median demographics were determined: 26 years of age (range 18–37 years), 23 weeks of gestational age at PMTCT enrollment (range 9–36 weeks), 7 years of education duration (range 0–12 years), 30 minutes travel time to KDH (range 0–120 minutes), and 0 Tanzanian Shilling of travel costs to KDH (range 0–2000 Tanzanian Shilling). Seven women (5.7%) were counseled and

Table 1. Summary of used adherence outcome measures.

Observation phase	Subgroup	Drugs	Adherence outcome measure
Antenatal	Maternal	AZT	Medication possession ratio from women having started prophylaxis in ANC
Intrapartum	Maternal	sdNVP	Intake ratio from all women observed intra/postpartumly
	Maternal	AZT/3TC	% of hours covered with drugs from total hospitalization hours between admission and delivery*
Postpartum	Maternal	AZT/3TC	• % of hours covered with drugs from total hospitalization hours between delivery and hospital discharge*
			• % of women having received their correct take-home dose from all women observed intra/postpartumly
	Infant	sdNVP	Intake ratio from all surviving newborns observed postpartumly
	Infant	AZT	• % of hours covered with drugs from total hospitalization hours between delivery and hospital discharge*
			• % of infants who received their correct take-home dose from all surviving infants observed postpartumly

*among hospital deliveries.

doi:10.1371/journal.pone.0021020.t001

tested together with their partner. Women's median CD4-cell count reached 391 cells/µl (range 200–883 cells/µl).

Forty-seven of 122 participants (38.5%) were lost to follow-up. Among these were 24 women who declined prophylaxis from the beginning, while 23 started prophylaxis, but failed to return to KDH. One woman had an abortion after starting prophylaxis and one baby died shortly after delivery. Seventy-three mother-child pairs were observed from the point of maternal PMTCT enrollment to the observation endpoint of maternal and infant drug supply in the intra/postpartum period. Figure 1 shows an overview of all undertaken analyses in different subgroups and respective drop-outs.

Factors associated with declining combination prophylaxis

Out of the 122 enrolled women, 35 (28.7%) declined pre-delivery prophylaxis. From these decliners, 11 returned to KDH in the intra/postpartum period.

In bivariate analysis, variables associated with declining pre-delivery prophylaxis were women's age of 23 years or below ($P<0.001$), no income-generating activity ($P = 0.006$), and a gestational age at enrollment of 24.5 weeks or below ($P<0.001$). In multivariate analysis, these variables showed to be associated independently with the decline of prophylaxis with P values of 0.002, 0.015, and 0.001, respectively. As shown in Table 2, other sociodemographic, economic and health-related variables, like education, travel distance or CD4 cell count, were not significantly associated with decline.

AZT-adherence levels before delivery

From 87 observed women having accepted prophylaxis, one subsequently had an abortion and was excluded from analysis (Figure 1). Among the remaining 86, the mean AZT-adherence before delivery was 77%. 56 women (65.1%) attained at least 80% adherence, and 43 women (50.0%) reached at least 95% adherence (Figure 2). Full adherence until delivery was achieved by 33 women (38.4%) who collected all AZT doses as required.

Disclosure of the HIV-status to the partner, a relative or a friend was positively associated with adherence rates before delivery: out of the 86 women who had started to take prophylaxis, 6 (7.0%) had not disclosed their HIV status and were significantly less (median 22.7%) adherent than women who had disclosed their status (median 97.3%, $P = 0.004$). Other variables were not significantly correlated with adherence levels (Table 3).

Overall, 53 out of 86 participants (61.6%) were not fully adherent, i.e. had missed at least one drug collection episode. In 34 of these (64.2%), reasons for failure in drug collection were unknown, partly because they did not state any reason when asked (11 women), and partly because they could not be asked due to loss to follow-up (23 women). Among the 19 women who had stated one ore more reasons (24 reasons mentioned altogether) for occurred missed drug collection episodes, personal reasons were mentioned most frequently (14/19 women, 73.7%), and included family obligations (mentioned by 6 women), forgetting appointments (mentioned by 4), transportation difficulties (mentioned by 3), and incorrect dosing of tablets (one mention). At the same time, 10 of the 19 women (52.6%) named hospital-based reasons, i.e. incorrectly indicated dates for the next visit (five mentions), neglected dispensation of drugs at KDH pharmacy (three mentions), or staff had decided to interrupt prophylaxis (two mentions).

Adherence during and after delivery

Out of 122 study participants, 74 were being observed in the intra/postpartum period (Figure 1). From these, 63 (85.1%) had accepted pre-delivery prophylaxis, while 11 (14.9%) women had declined. Pre-delivery prophylaxis acceptance was significantly associated with returning to KDH during or within 72 hours after delivery ($P<0.001$).

Sixty-four of 74 intra/postpartum observed participants (86.5%) delivered in KDH, and 10 women (13.5%) delivered at home but returned to KDH within 72 hours postpartum.

SdNVP was taken by 67 out of the 74 women (90.5%) in the correct time frame before delivery, 59 of these delivered in KDH, and eight delivered at home. Sixty-nine newborns (93.2%) received postnatal sdNVP in KDH, including 60 (93.8%) out of 64 KDH deliveries and 9 (90.0%) out of 10 home deliveries.

Regarding intrapartum AZT and 3TC, of the 64 deliveries in KDH, 38 women (59.4%) attained at least 80% and 27 (42.2%) at least 95% adherence. 26 women (40.6%) remained below 80% adherence. Between the time of delivery and hospital discharge, 34 of the 64 women (53.1%) reached 80% and 24 (37.5%) reached 95% adherence. From the 63 surviving newborns, for 32 (50.8%) at least 80% adherence was achieved, and for 12 children (19.0%) at least 95% adherence was achieved when starting the postpartum AZT tail during hospitalization.

From the 74 women which had either delivered in KDH or returned there within 72 hours, 58 (78.4%) received the correct amount of AZT and 3TC-tablets to take home, and 59 infants (80.8% from 73 surviving) were dispensed the correct take-home amount of AZT syrup. Achieved intra/postpartum adherence levels for mothers and infants are depicted in Figure 2.

Overall adherence before, during and after delivery

Initially, 122 women had agreed to take combination prophylaxis. One had an abortion after starting prophylaxis and one child died shortly after delivery. Out of the remaining 120 mother-child pairs, 10 (8.3%) achieved at least 80% adherence rates in all phases before, during and after delivery, and only one single mother-child pair (0.8%) achieved a 95% adherence level for the entire PMTCT intervention.

Discussion

Our observational study in a rural setting in Tanzania found relatively low levels of adherence to combination prophylaxis in all different PMTCT intervention phases.

Within the study population, young age and no income-generating activity of the women were found to increase the risk of not starting combination prophylaxis before delivery. This is in accordance with other studies, which identified similar risk factors to influence the acceptance of ART [25] and of sdNVP-based interventions [19]. Gestational age below 24 weeks at PMTCT enrollment was discovered as an additional risk factor for declining combination prophylaxis. An explanation for this could be the time gap emerging for women who become enrolled in an early pregnancy stage, but then have to wait until gestational week 28 until they can actually start prophylaxis. This time gap before drug intake might imply that antiretroviral prophylaxis has no acute priority, and forgetting future appointments may be likely. In response to this problem, revised WHO-guidelines of July 2010 recommend prophylaxis start from gestational week 14 onwards, aspiring to avoid idle time between enrollment and first drug intake [15]. Although this requires adhering to antenatal AZT for an even longer period of time, facilitating immediate prophylaxis start for the case of early PMTCT enrollment will simplify the PMTCT procedure to some extent and should be transferred into national guidelines as soon as possible. Yet, it will be of high importance to

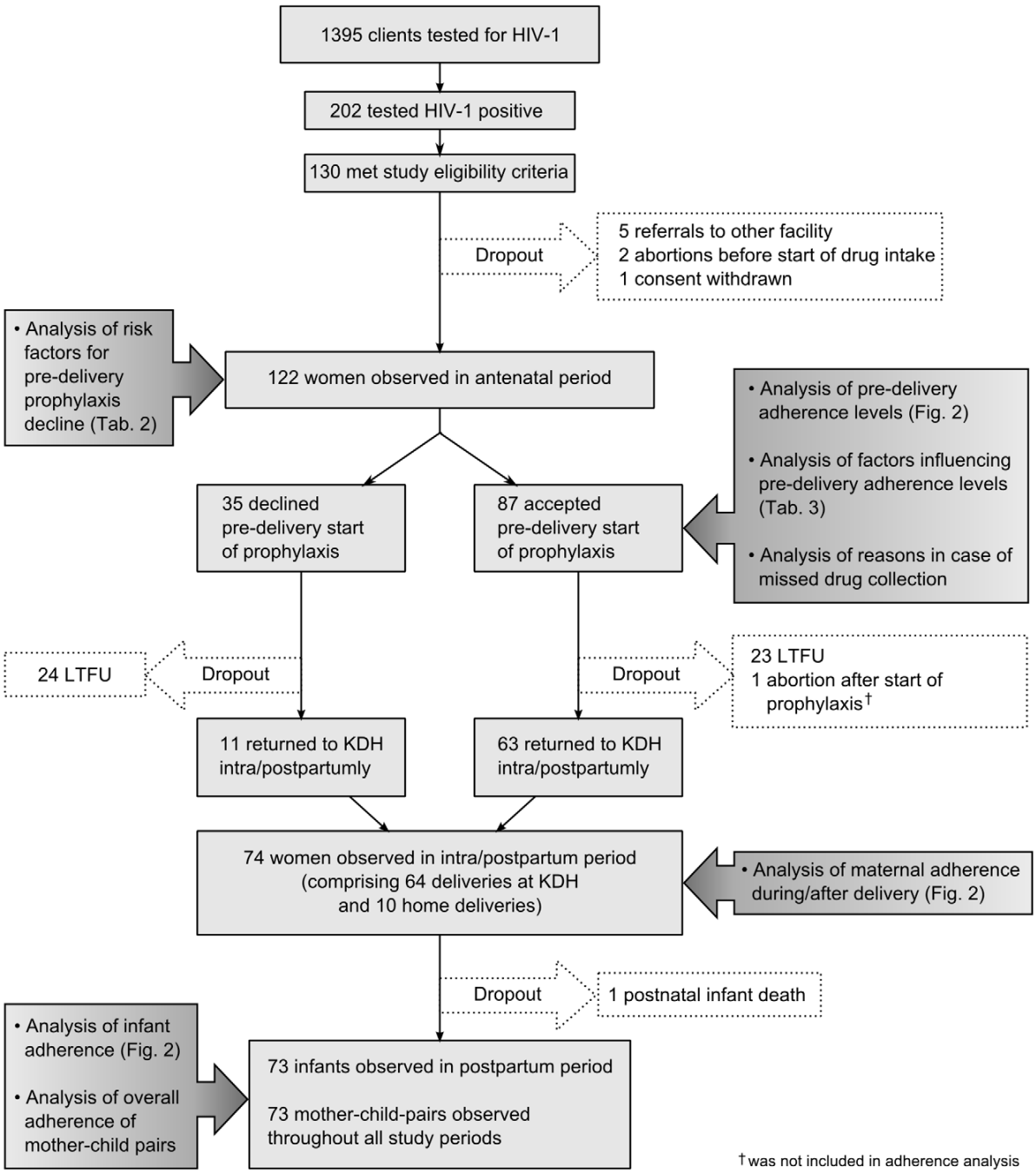

Figure 1. Profile of cohort and analyses. This figure is illustrating the formation of the cohort and of its different subgroups, including women observed in the antenatal period, women accepting/declining antenatal prophylaxis, women lost to follow-up (LTFU), women observed intra/postpartumly and infants observed postpartumly. It is indicated which analyses have been performed within the respective subgroups and where the results of those analyses are shown.
doi:10.1371/journal.pone.0021020.g001

keep track of possibly increased resistance formation arising from extended antenatal AZT monotherapy.

Among those who started combination prophylaxis, every third woman remained below an 80% adherence level in the period before delivery. This rate underachieves results of a similar study on ART adherence in a Zambian cohort of patients receiving treatment for their own health, where only about 15% of patients remained below an 80% adherence level [24]. In a previous meta-analysis on ART adherence among general population receiving treatment, it was reported that in 20 out of 22 studies, more than half of the patients met the thresholds of 95% or full adherence [26]. While those ART-related studies suggest that higher

Table 2. Risk factors for decline of pre-delivery prophylaxis.

			Bivariate					Mulitvariate	
Variable	**n**	**decline**	**OR**	**0.95 CI**		**P Chi²**		**AOR**	**P**
Age‡	**122**								
>23 years	91	17.58%	1.000					1.000	
≤23 years	31	61.29%	7.422	3.011–	18.292	<0,001	*	5.841	0.002
Education	**113**								
<7 years	19	21.05%	1.000						
≥7 years	94	28.72%	1.511	0.460–	4.967	0.494			
Income-gen. activity	**116**								
Yes	44	13.64%	1.000					1.000	
No	72	37.50%	3.800	1.420–	10.169	0.006	*	4.399	0.015
Marital status	**114**								
Single	24	25.00%	1.000						
Married	90	28.89%	1.182	0.422–	3.308	0.750			
Gravida	**122**								
Multigravida	113	28.32%	1.000						
Primigravida	9	33.33%	1.266	0.298–	5.369	0.719	**		
Gestational age‡	**122**								
>24.42 weeks	52	9.62%	1.000					1.000	
≤24.42 weeks	70	42.86%	7.050	2.501–	19.874	<0,001	*	7.820	0.001
Last delivery	**98**								
At home	27	29.63%	1.000						
At health facility	71	32.39%	1.138	0.434–	2.984	0.793			
Travel distance	**121**								
≤30 minutes	64	26.56%	1.000						
>30 minutes	55	32.73%	1.345	0.610–	2.965	0.462			
Transport costs	**117**								
No	74	28.38%	1.000						
Yes	43	30.23%	1.094	0.480–	2.493	0.831			
CD4 cell count	**122**								
>350	50	26.00%	1.000						
≤350	72	30.56%	1.252	0.559–	2.806	0.584			

OD = odds ratio; CI = confidence interval; AOR = adjusted odds ratio.
‡Threshold calculated with software JRip.
*Variables included into multivariate analyses.
**Fisher's exact test.
doi:10.1371/journal.pone.0021020.t002

adherence levels are achievable also in resource-limited settings, general ART programs can only represent an approximate comparative value with regards to PMTCT, because the subgroup of pregnant women involves specific implications for adherence. More research, filling the existing knowledge gap on adherence to PMTCT combination prophylaxis, is desirable to allow for putting our findings into context.

In our study, the only factor significantly influencing levels of pre-delivery adherence was HIV-status disclosure to the partner, a relative or a friend. Status disclosure is known to relieve women from emotional stress and to enable them to receive psychological and material support by their social environment [27]. Especially in rural settings, disclosure can facilitate issues like obtaining approval from the husband as the main decision maker to seek healthcare, and transportation to the hospital. From women who started to take prophylaxis, 93% disclosed their status, a high rate compared to other studies [28,29]. However, disclosure information could only be obtained from women who returned to ANC at least once after having been HIV-tested. Given that prophylaxis start is positively influenced by disclosure [29], our rate is likely to be overestimated due to the exclusion of 35 women declining prophylaxis.

Couple counseling and HIV-testing often facilitates partner disclosure and positively influences general uptake of PMTCT programs [30,31]. Deplorably few of our study participants were counseled and tested together with their partner, confirming the prevalent observation that adequate male involvement in PMTCT is still largely missing [32]. A stronger implementation of couple counseling and testing, and encouraging pregnant women to seek support in their social environment through status disclosure, not only to partners, but also to other confidants, e.g. in the form of treatment buddies or community groups, could be effective strategies for achieving better adherence rates [33,34].

Failure in pre-delivery adherence stands for women's missed or delayed hospital visits to collect drugs, which has been described as

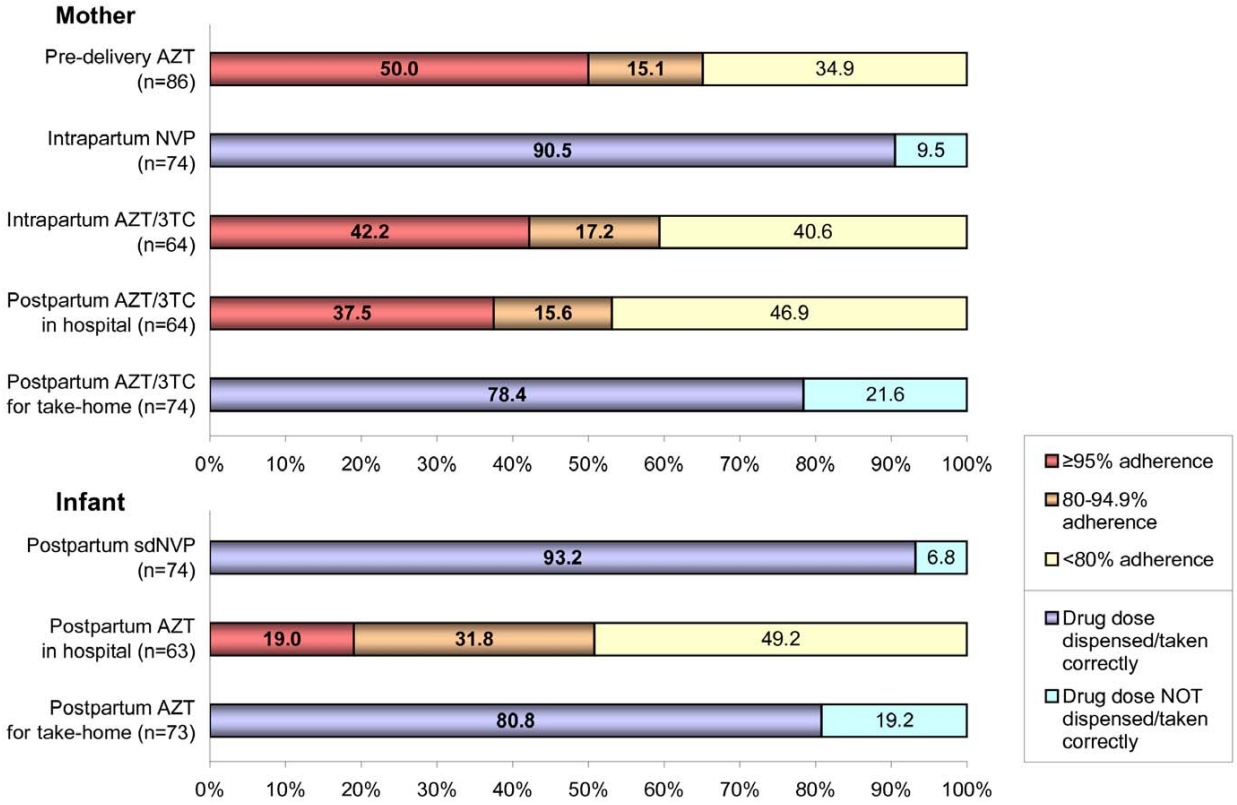

Figure 2. Adherence levels reached for different drugs in antenatal, intrapartum and postpartum period. Adherence cut-off levels of 95% and 80% are described for women's adherence to antenatal AZT, intrapartum AZT+3TC and postpartum AZT+3TC during hospitalization, as well as for infant adherence to AZT during hospitalization. For pre-delivery AZT intake, an adherence level of ≥95% was reached by 50.0% and a level of ≥80% was reached by 65.1% of women. For intrapartum AZT+3TC, adherence of ≥95% was reached by 42.2% of women who delivered in KDH and adherence of ≥80% was reached by 59.4% of them. For postpartum AZT+3TC during hospitalization, adherence of ≥95% was reached by 37.5% and adherence of ≥80% was reached by 53.1% of hospital-delivering women. In newborns, AZT adherence during hospitalization was ≥95% in 19.0% and ≥80% in 50.8% of the infants. For sdNVP, proportions reflect self-reported correct intake of drug doses for mothers (90.5%) and staff-reported correctly dispensed drug doses for newborns (93.2%). Postpartum take-home doses were dispensed correctly to 78.4% of women and 80.8% of infants.
doi:10.1371/journal.pone.0021020.g002

one of several causes for maladherence in other studies based on self-reported adherence rates to ART among general population receiving treatment for their own health [35,36]. We do not know the reasons for missed drug collection episodes in the majority of our study participants, either because they did not return to be asked, or because they did not want to state reasons. In those who shared their reasons for failure in collecting drugs, personal obstacles were mentioned most frequently, but every second of those women also stated that she failed to get drugs because of a wrongly scheduled date for the next drug collection or reluctance of drug dispensation by KDH staff. This demonstrates the crucial importance of high quality training of hospital staff [37].

Staff were also responsible for providing correct doses of AZT and 3TC at the right time during hospitalization of mother and newborn. For only about half of the mothers and babies, at least 80% adherence was achieved. The threshold of 95% adherence was met for less than half of the mothers and only one fifth of the babies. At hospital discharge, only about 80% of mothers and babies received the right amount of take-home drugs. These intra/postpartum adherence levels indicate suboptimal staff performance, and might partly be explained by the increased burden of work due to the introduction of combination prophylaxis. High workload can decrease motivation and dedication of staff, and might result in inadequate care. Systems of rotating staff members between departments often cause additional shortage and require continuous training of new PMTCT staff [37,38]. Reducing staff rotation and ensuring a close professional supervision could mitigate the shortage of well-trained personnel, and thereby considerably contribute to improved adherence rates during hospitalization. At the same time, it should be noted that in some other African countries, e.g. Kenya or Malawi, national guidelines recommend a simplified intrapartum regimen, administrating one dose of 600 mg AZT or 300 mg AZT twice daily instead of the three-hourly dosing throughout labor [39,40], a practice which could also reduce intrapartum care-related staff burden to some extent.

More than one third of the enrolled women were lost to follow-up, which is a larger fraction than in comparable studies on ART and sdNVP adherence [41,42]. Notably, only 10 mother-child pairs achieved at least 80% adherence in all phases of combination prophylaxis, and only one pair was adherent on a 95% level. We have targeted adherence levels of 80% and 95% for best comparability with other findings, but in fact there is a lack of evidence on necessary adherence levels to ensure the effectiveness of combination prophylaxis and to prevent emergence of resistant viral

Table 3. Influence of sociodemographic, economic and health-related factors on pre-delivery AZT adherence level.

Variable		Antenatal adherence	
	n	median	P*
Age	**86**	**93.92%**	0.558
≤23 years	11	95.08%	
>23 years	75	90.74%	
Education	**81**	**96.84%**	0.320
<7 years	15	87.16%	
≥7 years	66	97.86%	
Income-generating activity	**82**	**93.92%**	0.856
Yes	38	90.17%	
No	44	97.26%	
Marital status	**84**	**95.40%**	0.848
Single	18	93.92%	
Married	66	96.28%	
Gravida	**86**	**93.92%**	0.834
Primigravida	6	95.40%	
Multigravida	80	90.68%	
Gestational age	**86**	**93.92%**	0.176
≤24.42 weeks	47	87.76%	
>24.42 weeks	39	98.11%	
Last delivery	**66**	**95.96%**	0.278
At home	19	87.16%	
At health facility	47	96.84%	
Travel distance	**83**	**95.08%**	0.788
≤30 minutes	46	90.68%	
>30 minutes	37	97.78%	
Transport costs	**83**	**95.71%**	0.728
Yes	30	97.86%	
No	53	90.63%	
CD4 cell count	**86**	**93.92%**	0.304
≤350	36	98.48%	
>350	50	89.59%	
Disclosure	**86**	**93.92%**	0.004
Stated	80	97.26%	
Not stated	6	22.69%	

*Mann-Whitney U-Test.

doi:10.1371/journal.pone.0021020.t003

strains. In PMTCT regimen trials like the Kesho Bora study, detailed adherence levels among the study population have generally not been determined, while the trials have rather considered percentages of women who reached full adherence [43], or, like in the DITRAME trial, it was only assessed how many women met a certain threshold like 80% adherence [44]. Further research on specific necessary thresholds would be highly desirable for a more revealing interpretation concerning implications of adherence levels on drug effectiveness and resistance formation.

In the near future, the Tanzanian government will very likely align national PMTCT guidelines with WHO's 2010 revised recommendations, including the extension of ART-eligibility to those women with a CD4 count below 350 instead of 200, and including ARV intake throughout the entire breastfeeding period for infant (option A) or mother (option B) [15]. This will imply new challenges with regards to adherence, particularly for women choosing to breastfeed. According to WHO, 96.4% of Tanzanian infants are ever breastfed, with median breastfeeding duration of 21.1 months [45]. This is in compliance with PMTCT monitoring data from Mbeya Region, where over 95% of HIV positive women preferred breastfeeding to formula feeding (2009, data unpublished). Hence, for a mainly breastfeeding population like in our study setting, the chosen regimen option recommended in subsequent Tanzanian guidelines will be a highly relevant issue in the upcoming years.

An important limitation of the study is that pre-delivery adherence values in our analysis are based on MPRs. Although found to be a valid measurement for the reduction of viral load [46,47], MPRs bear the risk of overestimating real adherence values [48,49], because the amount of handed-out drugs might differ from the amount of actually ingested drugs. Suggesting that fewer women than assumed might actually have taken drugs as required, this would, however, strengthen our finding of suboptimal adherence.

Although we have chosen a representative district hospital strictly oriented in national guidelines, local customs might cause a systematical bias, and further research is strongly needed to assess the validity of our findings for other regions.

In conclusion, achieving high adherence rates during all phases of PMTCT combination prophylaxis has shown to be challenging in the rural setting of this study. Prophylaxis uptake might be improved by preponing drug intake to an earlier gestational age. Our findings underline the need for additional training and supervision for overburdened PMTCT staff as well as close supervision for PMTCT clients, especially by encouraging them to seek social support through status disclosure.

Combination prophylaxis for PMTCT is gradually replacing less effective regimens in many resource-limited regions of the world. However, the finding that only one mother-child pair managed to receive 95% of the intended quantity of drugs in all PMTCT phases in our study site results in a serious derogation of the assumed high effectiveness of combination prophylaxis in rural settings. It should be considered as a liability of health authorities to take limited structural conditions into account when planning, implementing and expanding combination prophylaxis for PMTCT.

Acknowledgments

The authors would like to express their gratitude to all staff and clients of Kyela District Hospital, the District Medical Officer, the Regional Health Management Team and the National AIDS Control Programme in Tanzania.

Author Contributions

Conceived and designed the experiments: IK JS AK FD JZ BJ-H GH ST. Performed the experiments: IK JS FD JZ. Analyzed the data: IK JS AK ST GH. Contributed reagents/materials/analysis tools: IK AK JS FD BJ-H. Wrote the paper: IK JS AK GH ST. Revision of masuscript for intellectual content: IK JS AK FD JZ BJ-H GH ST. Final approval: IK JS AK FD JZ BJ-H GH ST.

References

1. Joint United Nations Program on HIV/AIDS (UNAIDS) (2010) Global Report: UNAIDS report on the global AIDS epidemic 2010. WHO Library Catalogue-in-Publication Data. Available: http://www.unaids.org/globalreport/documents/20101123_GlobalReport_full_en.pdf. Accessed 10 April 2011.

2. De Cock KM, Fowler MG, Mercier E, de Vincenzi I, Saba J, et al. (2000) Prevention of mother-to-child HIV transmission in resource-poor countries: Translating research into policy and practice. JAMA 283: 1175–1182. doi:10.1001/jama.283.9.1175.

3. Guay LA, Musoke P, Fleming T, Bagenda D, Allen M, et al. (1999) Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomised trial. Lancet 354: 795–802.
4. Stringer EM, Sinkala M, Stringer JSA, Mzyece E, Makuka I, et al. (2003) Prevention of mother-to-child transmission of HIV in Africa: successes and challenges in scaling-up a nevirapine-based program in Lusaka, Zambia. AIDS 17: 1377–1382. doi:10.1097/01.aids.0000060395.18106.80.
5. Dabis F, Bequet L, Ekouevi DK, Viho I, Rouet F, et al. (2005) Field efficacy of zidovudine, lamivudine and single-dose nevirapine to prevent peripartum HIV transmission. AIDS 19: 309–318.
6. Lallemant M, Jourdain G, Le Coeur S, Kim S, Koetsawang S, et al. (2000) A trial of shortened zidovudine regimens to prevent mother-to-child transmission of human immunodefiency virus type 1. N Engl J Med 343: 982–991. doi:10.1056/NEJM200010053431401.
7. Maga G, Amacker M, Ruel N, Hbscher U, Spadari S (1997) Resistance to nevirapine of HIV-1 reverse transcriptase mutants: Loss of stabilizing interactions and thermodynamic or steric barriers are induced by different single amino acid substitutions. J Mol Biol 274: 738–747. doi:10.1006/jmbi.1997.1427.
8. Arrivé E, Newell ML, Ekouevii DK, Chaix ML, Thiebaut T, et al. (2007) Prevalence of resistance to nevirapine in mothers and children after single-dose exposure to prevent vertical transmission of HIV-1: A meta-analysis. Int J Epidemiol 36: 1009–1021. doi:10.1093/ije/dym104.
9. McIntyre JA, Hopley M, Moodley D, Eklund M, Gray GE, et al. (2009) Efficacy of short-course AZT plus 3TC to reduce nevirapine resistance in the prevention of mother-to-child HIV transmission: A randomized clinical trial. PLoS Med 6: e1000172. doi:10.1371/journal.pmed.1000172.
10. World Health Organization (WHO) (2006) Antiretroviral drugs for treating pregnant women and preventing HIV infection in infants: Towards universal access: Recommendations for a public health approach. 2006 version. Available: http://whqlibdoc.who.int/publications/2006/9789241594660_eng.pdf. Accessed 18 Sept 2010.
11. United Nations Development Programme (2008) Human Development Indices. Available: http://hdr.undp.org/en/media/HDI_2008_EN_Complete.pdf. Accessed 18 Sept 2010.
12. World Health Organization (2009) World Health Statistics. Available: http://www.who.int/whosis/whostat/EN_WHS09_Full.pdf. Accessed 18 Sept 2010.
13. Joint United Nations Program on HIV/AIDS (UNAIDS) (2004) United Republic of Tanzania: Epidemiological fact sheets on HIV/AIDS and sexually transmitted infections. Available: http://data.unaids.org/publications/Fact-Sheets01/tanzania_en.pdf. Accessed 18 Sept 2010.
14. The United Republic of Tanzania, Ministry of Health and Social Welfare (2007) Prevention of Mother-To-Child Transmission of HIV (PMTCT), National Guidelines. Dar es SalaamTanzania: Ministry of Health and Social Welfare.
15. World Health Organization (WHO) (2010) Antiretroviral drugs for treating pregnant women and preventing HIV infection in infants: Recommendations for a public health approach. 2010 version. Geneva, Switzerland: WHO Press, Available: http://whqlibdoc.who.int/publications/2010/9789241599818_eng.pdf. Accessed 29 July 2010.
16. Gross R, Bilker WB, Friedman HM, Strom BL (2001) Effect of adherence to newly initiated antiretroviral therapy on plasma viral load. AIDS 15: 2109–2117.
17. Garcia PM, Kalish LA, Pitt J, Minkoff H, Quinn TC, et al. (1999) Maternal levels of plasma human immunodeficiency virus type 1 RNA and the risk of perinatal transmission. N Engl J Med 341: 394–402. doi:10.1056/NEJM199908053410602.
18. Smith R (2006) Adherence to antiretroviral HIV drugs: How many doses can you miss before resistance emerges? Proc Biol Sci 273: 617–624. doi:10.1098/rspb.2005.3352.
19. Karcher H, Kunz A, Poggensee G, Mbezi P, Mugenyi K, et al. (2006) Outcome of different nevirapine administration strategies in preventing mother-to-child transmission (PMTCT) programs in Tanzania and Uganda. J Int AIDS Soc 8: 12. doi:10.1186/1758-2652-8-2-12.
20. Tanzania Commission for AIDS (TACAIDS), National Bureau of Statistics (NBS), and ORC Macro (2008) Tanzania HIV & Malaria Indicator Survey 2007 Preliminary Report. Available: http://www.ihi.or.tz/docs/Tz{HIV}-MalariaIndicatorSurvey-0708-PreliminaryReport.pdf. Accessed 18 Sept 2010.
21. World Health Organization (2007) Chronik HIV Care with ARV Therapy and Prevention. Available: http://www.who.int/hiv/pub/imai/Chronic_HIV_Care7.05.07.pdf. Accessed 18 Sept 2010.
22. Sikka R, Xia F, Aubert RE (2005) Estimating medication persistency using administrative claims data. Am J Manag Care 11: 449–457.
23. Hall M, Frank E, Holmes G, Pfahringer B, Reutemann P, et al. (2009) The WEKA data mining software: An update. SIGKDD Explorations 11: 10–18. Software available: http://www.cs.waikato.ac.nz/~ml/weka/. Accessed 18 Sept 2010.
24. Chi BH, Cantrell RA, Zulu I, Mulenga LB, Levy JW, et al. (2009) Adherence to first-line antiretroviral therapy affects non-virologic outcomes among patients on treatment for more than 12 months in Lusaka, Zambia. Int J Epidemiol 38: 746–756. doi:10.1093/ije/dyp004.
25. Byakika-Tusiime J, Oyugi JH, TumwikirizeWA, Katabira ET, Mugyenyi PN, et al. (2005) Adherence to HIV antiretroviral therapy in HIV+ Ugandan patients purchasing therapy. Int J STD AIDS 16: 38–41. doi:10.1258/0956462052932548.
26. Mills EJ, Nachega JB, Buchan I, Orbinski J, Attaran A, et al. (2006) Adherence to antiretroviral therapy in sub-Saharan Africa and North America: A meta-analysis. JAMA 296: 679–690. doi:10.1001/jama.296.6.679.
27. Norman A, Chopra M, Kadiyala S (2007) Factors related to HIV disclosure in 2 South African communities. Am J Public Health 97: 1775–1781. doi:10.2105/AJPH.2005.082511.
28. Brou H, Djohan G, Becquet R, Allou G, Ekouevi DK, et al. (2007) When do HIV-infected women disclose their HIV status to their male partner and why? A study in a PMTCT programme, Abidjan. PLoS Med 4: e342. doi:10.1371/journal.pmed.0040342.
29. Maman S, Medley A (2004) Gender dimensions of HIV status disclosure to sexual partners: Rates, barriers and outcomes: A review paper. Available: http://www.who.int/gender/documents/en/genderdimensions.pdf. Accessed 29 July 2010.
30. Conkling M, Shutes EL, Karita E, Chomba E, Tichacek A, et al. (2010) Couples' voluntary counselling and testing and nevirapine use in antenatal clinics in two African capitals: A prospective cohort study. J Int AIDS Soc 13: 10. doi:10.1186/1758-2652-13-10.
31. Farquhar C, Kiarie JN, Richardson BA, Kabura MN, John FN, et al. (2004) Antenatal couple counseling increases uptake of interventions to prevent HIV-1 transmission. J Acquir Immune Defic Syndr 37: 1620–1626.
32. Theuring S, Mbezi P, Luvanda H, Jordan-Harder B, Kunz A, et al. (2009) Male involvement in PMTCT services in Mbeya Region, Tanzania. AIDS Behav 13 Suppl 1: 92–102. doi:10.1007/s10461-009-9543-0.
33. Wouters E, Van Damme W, van Rensburg D, Meulemans Ha (2008) Impact of baseline health and community support on antiretroviral treatment outcomes in HIV patients in South Africa. AIDS 22: 2545–2548. doi:10.1097/QAD.0b013e32831c5562.
34. Sarna A, Weiss E (2006) Current research and good practice in HIV and AIDS treatment education. Available: http://unesdoc.unesco.org/images/0014/001497/149722e.pdf. Accessed 18 Sept 2010.
35. Nachega JB, Stein DM, Lehman DA, Hlatshwayo D, Mothopeng R, et al. (2004) Adherence to antiretroviral therapy in HIV-infected adults in Soweto, South Africa. AIDS Res Hum Retroviruses 20: 1053–1056. doi:10.1089/aid.2004.20.1053.
36. Mugusi F, Mugusi S, Bakari M, Hejdemann B, Josiah R, et al. (2009) Enhancing adherence to antiretroviral therapy at the HIV clinic in resource constrained countries; The Tanzanian experience. Trop Med Int Health 14: 1226–1232.
37. Nuwagaba-Biribonwoha H, Mayon-White RT, Okong P, Carpenter LM (2007) Challenges faced by health workers in implementing the prevention of mother-to-child HIV transmission (PMTCT) programme in Uganda. J Public Health 29: 269–274. doi:10.1093/pubmed/fdm025.
38. Levy JM (2009) Women's expectations of treatment and care after an antenatal HIV diagnosis in Lilongwe, Malawi. Reprod Health Matters 17: 152–161. doi:10.1016/S0968-8080(09)33436-9.
39. Government of Malawi, Ministry of Health (2008) Prevention of mother to child transmission of HIV and paediatric HIV care guidelines. Second Edition, July 2008. Government of Malawi. Available: http://www.aidstar-one.com/sites/default/files/PMTCT%20Guidelines%20Malawi%202008.pdf. Accessed 10 April 2011.
40. Government of Kenya, Ministry of Health (2009) Guidelines for prevention of mother to child transmission (PMTCT) of HIV/AIDS in Kenya. Third Edition, 2009. Available: http://www.aidstar-one.com/sites/default/files/PMTCT%20Guidelines%20Kenya%202009.pdf. Accessed 10 April 2011.
41. Karcher H, Omondi A, Odera J, Kunz A, Harms G (2007) Risk factors for treatment denial an d loss to follow-up in an antiretroviral treatment cohort in Kenya. Trop Med Int Health 12: 687–694.
42. Tonwe-Gold B, Ekouevi DK, Viho I, Amani-Bosse C, Toure S, et al. (2007) Antiretroviral treatment and prevention of peripartum and postnatal HIV transmission in West Africa: Evaluation of a two-tiered approach. PLoS Med 4: e257. doi:10.1371/journal.pmed.0040257.
43. The Kesho Bora Study Group (2011) Triple antiretroviral compared with zidovudine and single-dose nevirapine prophylaxis during pregnancy and breastfeeding for prevention of mother-to-child transmission of HIV-1 (Kesho Bora study): A randomised controlled trial. Lancet Infect Dis 11: 171–180.
44. Meda N, Leroy V, Viho I, Msellati P, Yaro S, et al. (2002) Field acceptability and effectiveness of the routine utilization of zidovudine to reduce mother-to-child transmission of HIV-1 in West Africa.. AIDS 16: 2323–2328.
45. WHO Global Data Bank on Infant and Young Child Feeding (IYCF) (updated 2009) Infant and young child feeding data by country, Tanzania. National Bureau of Statistics (NBS) and ORC Macro. Tanzania Demographic and Health Survey 2004–05. Available: http://www.who.int/nutrition/databases/infantfeeding/countries/tza.pdf. Accessed April 10 2011.
46. Grossberg R, Zhang Y, Gross R (2004) A time-to-prescription-refill measure of antiretroviral adherence predicted changes in viral load in HIV. J Clin Epidemiol 57: 1107–1110. doi:10.1016/j.jclinepi.2004.04.002.
47. Weidle PJ, Wamai N, Solberg P, Liechty C, Sendagala S, et al. (2006) Adherence to antiretroviral therapy in a home-based AIDS care programme in rural Uganda. Lancet 368: 1587–1594. doi:10.1016/S0140-6736(06)69118-6.
48. Gill CJ, Hamer DH, Simon JL, Thea DM, Sabin LL (2005) No room for complacency about adherence to antiretroviral therapy in sub-Saharan Africa. AIDS 19: 1243–1249.
49. Steiner J, Prochazka A (1997) The assessment of refill compliance using pharmacy records: Methods, validity, and applications. J Clin Epidemiol 50: 105–116. doi:10.1016/S0895-4356(96)00268-5.

Druckexemplar Publikation 2

Hauser A, Sewangi J, Mbezi P, Dugange F, Lau I, Ziske J, Theuring S, Kuecherer C, Harms G, Kunz A

Emergence of Minor Drug-Resistant HIV-1 Variants after Triple Antiretroviral Prophylaxis for Prevention of Vertical HIV-1 Transmission

PLoS ONE 2012;7(2):e32055

doi:10.1371/journal.pone.0032055

OPEN ACCESS Freely available online

PLoS one

Emergence of Minor Drug-Resistant HIV-1 Variants after Triple Antiretroviral Prophylaxis for Prevention of Vertical HIV-1 Transmission

Andrea Hauser[1,2]*, Julius Sewangi[3], Paulina Mbezi[4], Festo Dugange[5], Inga Lau[1], Judith Ziske[1], Stefanie Theuring[1], Claudia Kuecherer[2], Gundel Harms[1], Andrea Kunz[1]

1 Institute of Tropical Medicine and International Health, Charité – Universitätsmedizin Berlin, Berlin, Germany, **2** Center for HIV and Retrovirology, Robert Koch-Institute, Berlin, Germany, **3** Regional AIDS Control Program Mbeya Region, Ministry of Health and Social Welfare, Dar es Salaam, Tanzania, **4** PMTCT Service Mbeya Region, Ministry of Health and Social Welfare, Dar es Salaam, Tanzania, **5** Kyela District Hospital, Ministry of Health and Social Welfare, Dar es Salaam, Tanzania

Abstract

Background: WHO-guidelines for prevention of mother-to-child transmission of HIV-1 in resource-limited settings recommend complex maternal antiretroviral prophylaxis comprising antenatal zidovudine (AZT), nevirapine single-dose (NVP-SD) at labor onset and AZT/lamivudine (3TC) during labor and one week postpartum. Data on resistance development selected by this regimen is not available. We therefore analyzed the emergence of minor drug-resistant HIV-1 variants in Tanzanian women following complex prophylaxis.

Method: 1395 pregnant women were tested for HIV-1 at Kyela District Hospital, Tanzania. 87/202 HIV-positive women started complex prophylaxis. Blood samples were collected before start of prophylaxis, at birth and 1–2, 4–6 and 12–16 weeks postpartum. Allele-specific real-time PCR assays specific for HIV-1 subtypes A, C and D were developed and applied on samples of mothers and their vertically infected infants to quantify key resistance mutations of AZT (K70R/T215Y/T215F), NVP (K103N/Y181C) and 3TC (M184V) at detection limits of $<$1%.

Results: 50/87 HIV-infected women having started complex prophylaxis were eligible for the study. All women took AZT with a median duration of 53 days (IQR 39–64); all women ingested NVP-SD, 86% took 3TC. HIV-1 resistance mutations were detected in 20/50 (40%) women, of which 70% displayed minority species. Variants with AZT-resistance mutations were found in 11/50 (22%), NVP-resistant variants in 9/50 (18%) and 3TC-resistant variants in 4/50 women (8%). Three women harbored resistant HIV-1 against more than one drug. 49/50 infants, including the seven vertically HIV-infected were breastfed, 3/7 infants exhibited drug-resistant virus.

Conclusion: Complex prophylaxis resulted in lower levels of NVP-selected resistance as compared to NVP-SD, but AZT-resistant HIV-1 emerged in a substantial proportion of women. Starting AZT in pregnancy week 14 instead of 28 as recommended by the current WHO-guidelines may further increase the frequency of AZT-resistance mutations. Given its impact on HIV-transmission rate and drug-resistance development, HAART for all HIV-positive pregnant women should be considered.

Citation: Hauser A, Sewangi J, Mbezi P, Dugange F, Lau I, et al. (2012) Emergence of Minor Drug-Resistant HIV-1 Variants after Triple Antiretroviral Prophylaxis for Prevention of Vertical HIV-1 Transmission. PLoS ONE 7(2): e32055. doi:10.1371/journal.pone.0032055

Editor: Fabrizio Mammano, INSERM, France

Received September 29, 2011; **Accepted** January 19, 2012; **Published** February 23, 2012

Funding: The work was supported by the German Ministry for Economic Cooperation and Development through project 01.2029.5 (Prevention of Mother-to Child Transmission of HIV) and by a grant of the H.W. & J. Hector Stiftung, Germany. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: andrea.hauser@charite.de

Introduction

Mother-to-child transmission of HIV-1 in resource-limited settings accounts for almost 16% of all new HIV-1 infections in Sub-Saharan Africa [1]. Antiretroviral drugs for HIV-1-infected pregnant women and their infants are an essential component in reducing mother-to-child transmission of HIV-1. The non-nucleoside reverse transcriptase inhibitor (NNRTI) nevirapine (NVP) has been widely applied as single dose (NVP-SD) prophylaxis at the onset of labor [2]. However, due to the low genetic barrier of NVP even a single dose frequently induces viral resistance [3–10], thus compromising the success of subsequent NNRTI-containing highly active antiretroviral treatment (HAART) if initiated within 6–12 month after prophylaxis [11–13]. To reduce viral resistance as well as to further lower the vertical transmission risk of HIV-1, the WHO guidelines for the prevention of mother-to-child transmission (PMTCT) of 2006 and 2010 [14,15] recommend complex antiretroviral prophylaxis. This is composed of antenatal zivoduvine (AZT) for three (2006) or six months (2010), NVP-SD at labor onset and AZT/lamivudine (3TC) during labor and for one week postnatally. In 2008, complex prophylaxis was recommended by the national Tanza-

nian PMTCT guidelines as preferred PMTCT regimen [16]. Monotherapy of antiretroviral drugs, however, inherently involves the risk of drug resistance development. Selection of AZT-resistant virus during prenatal AZT monotherapy might decrease the efficacy of future AZT-containing prophylactic and therapeutic regimens. Furthermore, as both NVP and 3TC rapidly select for drug-resistant virus, dual- or multi-resistant HIV-1 variants could emerge. Even minor drug-resistant HIV-1 variants representing small proportions of the total viral population can impair virological outcome of HAART [17–24]. Hence, it is mandatory to characterize the resistance development including minority species following complex prophylaxis, which to our knowledge has not been assessed for the WHO-recommended complex prophylaxis regimen. The aim of this study was to evaluate the emergence of HIV-1 variants resistant against AZT, NVP and/or 3TC following complex antiretroviral prophylaxis in a rural district hospital in Kyela, Mbeya Region, Tanzania. For this purpose, we developed, evaluated and applied highly sensitive allele-specific PCR (ASPCR) assays enabling the detection and quantification of three key mutations for AZT resistance (K70R, T215Y and T215F), the two most common NVP-associated resistance mutations (K103N and Y181C) and the most frequent 3TC-selected mutation M184V in the *pol* open reading frame with a detection limit of $<1\%$ [25,26]. ASPCR assays were adapted for HIV-1 subtypes A, C and D which are common in Sub-Saharan Africa and prevalent in Mbeya Region, Tanzania [27]. Subsequently, blood specimens from HIV-1-infected pregnant Tanzanian women and their vertically infected infants who had taken complex antiretroviral prophylaxis were analyzed.

Materials and Methods

Ethics Statement

Ethical approval was obtained from the local Mbeya Medical Research and Ethics Committee, the National Institute for Medical Research of Tanzania and the ethical committee of Charité – Universitätsmedizin Berlin in Germany. We obtained informed written consent from all participants involved in our study.

Clinical samples and study design

The present study analyzes the HIV-1 resistance development in HIV-1-infected Tanzanian women and their infants as part of an observational study at Kyela District Hospital, Mbeya Region between October 2008 and September 2009 [28]. In March 2008, complex antiretroviral prophylaxis was introduced as the standard PMTCT regimen at Kyela District Hospital. According to WHO PMTCT guidelines from 2006 [14] and National Tanzanian PMTCT guidelines [16], women were offered complex antiretroviral prophylaxis composed of AZT starting in gestational week 28 (2×300 mg per day), or as soon as possible thereafter, followed by NVP-SD (200 mg) at labor onset and AZT (300 mg) every three hours plus 3TC (150 mg) every 12 hours during labor, followed by a one week postpartum course of AZT (2×300 mg per day) and 3TC (2×150 mg per day). Infants received NVP-SD (2 mg/kg) within 72 hrs after birth and AZT (4 mg/kg per day) for one week. In case the mother had taken antenatal AZT for less than four weeks, the infant received postnatal AZT for four weeks. Blood samples were collected before start of AZT prophylaxis, during pregnancy, at delivery and at 1–2, 4–6, and 12–16 weeks postnatally.

202 of 1395 (14.5%) pregnant women tested for HIV-1 during antenatal care were HIV-1 positive. 122 HIV-positive women were included in the observational study as they fulfilled the following eligibility criteria: no HAART, no clinical or immunological indication to start HAART, i.e. CD4 cell count $>=200$ cells/mm3 and clinical categories A or B according to CDC classification, age $>=18$ years, absence of other severe diseases including psychiatric disorders, written informed consent [28]. Eventually, 87 of the 122 eligible women started AZT prophylaxis during pregnancy [28]. Women and if applicable their HIV-infected infants were included in the resistance analysis if they had taken AZT in pregnancy for at least two weeks, if they had taken NVP at labor onset, and if a delivery sample and at least two postnatal (1–2 weeks, 4–6 weeks and/or 12–16 weeks) plasma samples were available. In the case of home delivery, the last antenatal specimen was used as "delivery sample". Additionally, a baseline sample prior to AZT intake had to be amplifiable in order to establish an individual cut-off for resistance detection [29]. No woman received any other antiretroviral drugs during the study period. Children of the study cohort were breastfed.

Detection and quantification of drug-resistant HIV-1

Drug-resistant mutations in the *pol* open reading frame of HIV-1 were detected by ASPCR which is an established and widely used method for the analysis of minor drug-resistant HIV-1 variants [5,29–33]. The assay is composed of two consecutive real-time PCRs. The outer real-time PCR amplified a reverse transcriptase (RT) fragment comprising the codons of interest (codons 22 to 236 of the RT) and was also used for quantification of viral load. The inner ASPCR was composed of one real-time PCR reaction with discriminatory ability for mutant sequences using selective primers and one generic real-time PCR reaction amplifying both wild-type and mutant sequences using non-selective primers (Table 1). For each resistance mutation, an individual inner ASPCR assay had to be designed. In total, seven ASPCR assays were performed per sample: two AZT mutations confering high level resistance (T215Y, T215F) and one early AZT mutation (K70R) confering only low level resistance but indicating for emergence of AZT-resistance; additionally the two most common NVP-selected resistance mutations (K103N and Y181C) and the most frequent 3TC-selected mutation M184V were analysed [34,35] (details in Materials and Methods S1).

Vertical transmission of HIV-1

The HIV-status of newborns was determined by RT-PCR of blood specimens collected 4–6 weeks after birth using the above described outer PCR. Infants with a positive PCR result at 4–6 week were defined to be HIV-infected whereas infants with a negative PCR result were assumed to be not HIV-infected. If the 4–6 week sample was lacking, an earlier blood sample from delivery or week 1–2 was analysed. If the earlier sample was PCR-positive, the child was considered to be HIV-infected 4–6 weeks after birth as well; if the earlier blood sample was PCR-negative, the infant was excluded from calculation of transmission rate as the HIV status week 4–6 after birth could not be determined.

Population-based sequencing and determination of HIV-1 subtype

For population-based sequencing of the 644 bp product generated by outer PCR, the automated sequencer 3130xl Genetic Analyzer (Applied Biosystems, Darmstadt, Germany) and the HIV SEQ MIX B, D and G of the Viroseq HIV-1 Genotyping System version 2.0 (Abbott, Wiesbaden, Germany) were applied. To exclude sample mix-up and to confirm vertical HIV-1 transmission, phylogenetic analysis of maternal and infant sequences generated by population-based sequencing was performed using

Table 1. Oligonucleotide sequences of primers used in outer and allele-specific PCR (ASPCR).

Assay and primer name	Nucleotide sequence	Nucleotide position (HXB2)	Fragment size (bp)
Outer-PCR			
HIV-TZ FOR	5′- AAACAATGGCCATTRACAGARGA-3′+	2613–2635	
HIV-TZ REV	5′- GGATGGAGTTCATAICCCATCCA-3′−	3234–3256	644
K70R ASPCR			
TZ-K70 FOR 1	5′- GCIATAAARAARAARGACAGYACTC-3′+	2733–2757	
TZ-K70R FOR 2	5′- GCIATAAARAARAARGACAGYACTCG-3′+	2733–2758	
TZ-K70 REV	5′- CCCACATCYAGTACTGTYACTGATTT-3′−	2859–2884	152
K103N ASPCR			
TZ-K103 FOR	5′- GGCCTGAAAATCCATAYAAYACTCC-3′+	2701–2725	
TZ-K103 REV1	5′- CCCACATCYAGTACTGTYACTGATTT-3′−	2859–2884	
TZ-K103N(C) REV3	5′- CCCACATCYAGTACTGTYACTGATTGG-3′−	2858–2884	
TZ-K103N(T) REV4	5′- CCCACATCYAGTACTGTYACTGATTGA-3′−	2858–2884	184
Y181C ASPCR			
TZ-Y181/M184 FOR	5′- AAATCAGTRACAGTACTRGATGTRGG-3′+	2859–2884	
TZ-Y181 REV1	5′- ATCCTACATACAARTCATCCATRTATTGA-3′−	3092–3120	
TZ-Y181C REV3	5′- ATCCTACATACAARTCATCCATRTATTGCC-3′−	3091–3120	262
M184V ASPCR			
TZ-Y181/M184 FOR	5′- AAATCAGTRACAGTACTRGATGTRGG-3′+	2859–2884	
TZ-M184 REV1	5′- TCAGATCCTACATAYAARTCATCCA-3′−	3101–3124	
TZ-M184V REV3	5′- TCAGATCCTACATAYAARTCATCIGC-3′−	3098–3124	266
T215Y/F ASPCR			
TZ-T215 FOR	5′- CACAGGGATGGAAAGGATCACC-3′+	2998–3019	
TZ-T215 REV1	5′- CTTCTGATGYTTYTTGTCTGGIGT-3′−	3185–3205	
TZ-T215Y REV3	5′- CTGATGYTTYTTGTCTGGIGTCTA-3′−	3182–3205	
TZ-T215F REV4	5′- CTGATGYTTYTTGTCTGGIGTCAA-3′−	3182–3205	
TZ-T215F REV5	5′- CTGATGYTTYTTGTCTGGIGTTAA-3′−	3182–3205	208

doi:10.1371/journal.pone.0032055.t001

the neighbor joining method (Bioedit 7.0.9) [36]. HIV-1 subtyping of the *pol* sequence was performed using the REGA HIV-1 subtyping tool [37].

Statistical analysis

The non-parametric Mann-Whitney U test was used to assess significant differences between two independent samples whereas the Wilcoxon signed-rank test was used to analyze repeated measurements. Chi-Square test or Fisher's exact test were applied to analyze the independence of categorical variables. Testing of significant correlations between two continuous variables was done by Pearson's correlation coefficient. For descriptive analysis, median and interquartile ranges (IQR) were calculated. Two-sided tests were used and $p<0.05$ was considered statistically significant. Drug-resistant HIV-1 variants carrying the K103N (AAC) mutation and the K103N (AAT) mutation were summed to obtain the total proportion of virus carrying the K103N mutation. Statistical analysis was carried out using PASW Statistics 18 (SPSS Inc., Chicago, Illinois, USA).

Results

Sample characteristics

Of 87 women having started complex prophylaxis, 50 women fulfilled the eligibility criteria and were included in the resistance analysis, together with their seven vertically HIV-infected infants. Median baseline characteristics before start of prophylaxis were: age 28 years (IQR 26–30), HIV-1 viral load 1.25×10^4 copies/mL (IQR 4.4×10^3–4.5×10^4) and CD4 cell counts of 390 cells/mm^3 (IQR 260–492). The median maternal viral load was 2.9×10^3 copies/mL (IQR 1.4×10^3–6.8×10^3) at delivery, 1.7×10^3 copies/mL (IQR 1.3×10^3–5.8×10^3) 1–2 weeks postpartum, 1.2×10^4 copies/mL (IQR 6.3×10^3–3.7×10^4) 4–6 weeks postpartum and 2.5×10^4 copies/mL (IQR 1.2×10^4–3.7×10^4) 12–16 weeks postpartum. Compared to baseline viral load, maternal viral loads at delivery and 1–2 weeks postpartum were significantly lower (both $p<0.001$) but reached similar levels at 4–6 weeks ($p=0.45$) and at 12–16 weeks ($p=0.54$) postpartum, respectively. Women received AZT during pregnancy for a median of 53 days (IQR 39–64). Thirty-seven (74%) women delivered at Kyela District Hospital whereas 13 (26%) women delivered at home or in another health facility. Regardless of the place of delivery, all women took NVP-SD before birth. Thirty-four of 37 women who delivered at Kyela District Hospital received intrapartum AZT/3TC. Forty-one women took AZT/3TC postpartum for one week, while another five women took AZT but not 3TC postpartum. In total, 86% (43/50) of women took at least one dose of 3TC. Forty-four (88%) infants received NVP-SD after birth, including all 37 newborns born at Kyela District Hospital and 7/13 infants born at another place. Forty-five (90%) newborns took AZT postnatally; 42 of whom for one week and three for four weeks. Forty-nine of 50 infants including all HIV-infected infants were breastfed. 28%

(14/50) of the women were infected with HIV-1 subtype A1, 68% (34/50) with subtype C and two women (4%) with subtype D. None of the 50 baseline samples exhibited preexisting drug-selected mutations in the RT as determined by population sequencing.

Quantification of HIV-1 RNA by outer PCR

A standard curve was calculated from eight independent runs ($r^2 = 0.992$, standard deviation 0.004) by using defined concentrations of HIV-1 NL4.3 virus ranging from 6.5×10^1–10^7 copies/ml (details in Materials and Methods S1). The lower limit of detection for HIV-1 RNA was 650 copies/ml.

226 maternal samples (mean 4.5 samples per woman) were available, of which 211 were successfully amplified and quantified in the outer PCR, including 50/50 baseline samples, 48/50 delivery samples, 37/46 1–2 weeks samples (which displayed the lowest viral load), 47/49 4–6 weeks samples and 29/31 12–16 weeks samples. Out of the seven vertically HIV-1-infected newborns, 11/15 available samples were amplifiable in the outer PCR.

Evaluation of ASPCR assays

Accuracy, precision, sensitivity and specificity of ASPCR. Accuracy, precision and sensitivity (detection limit) of all ASPCR assays are shown in Table 2. The coefficient of variation as measurement of inter-assay precision did not exceed 47% (range 12%–47%, data not shown). The lower detection limit for evidence of minor drug-resistant HIV-1 variants was 0.99% for K70R, 0.04% for K103N (AAC), 0.01% for K103N (AAT), 0.35% for Y181C, 0.63% for M184V, 0.33% for T215Y and 0.42% for T215F (Table 2). Specificity for HIV-1 wild-type controls was 100% for all ASPCR assays.

Some maternal ASPCR results had to be excluded from analysis due to polymorphisms in primer binding sites (details in Materials and Methods S1); this affected two women for K103N analysis, one woman for Y181C analysis and six women for K70R analysis.

Detection limit for drug-resistant HIV-1 in samples with low viral load

The sensitivity of ASPCR assays for detection of drug-resistant HIV-1 correlates with the input viral load. In order to avoid false positive results, we established a threshold considering the respective viral load of any given sample (see Materials and Methods S1). The lower detection limit for drug-resistant HIV-1 variants was 0.17% for samples with 10^4 copies/ml and 0.97% for samples with 10^3 copies/ml. If the calculated proportion of drug-resistant HIV-1 fell below the calculated threshold, it was considered to be false positive and presence of HIV-1 wild type was assumed; this affected the detection of K103N and T215Y only once.

Emergence of drug-resistant HIV-1 variants in Tanzanian women

In total, 20/50 (40%) women exhibited drug-resistant virus during the observation period (Table 3), including 13/34 (38%) women infected with HIV-1 subtype C, 6/14 (43%) women with subtype A1 and 1/2 with subtype D. Genotypic mutations associated with decreased susceptibility to AZT were detected in 11/50 (22%) women (7/50 (14%) containing K70R alone and 4/50 (8%) with T215Y/F mutation) whereas 9/50 (18%) women harbored NVP-resistant virus (K103N and/or Y181C). In 4/50 (8%) women a 3TC-resistance mutation (M184V) was identified, of these 3/50 (6%) developed drug-resistant HIV-1 strains against more than one drug (Figure 1).

In 5/20 women, drug-resistant variants were already detectable at delivery and all of these women carried HIV-1 with AZT-selected resistance mutations only. In 4/20 women, resistant virus was detectable for the first time 1–2 weeks after delivery and in 11/20 women resistant variants were not present before weeks 4–6. 50% of the women with HIV-1 resistance still exhibited drug-resistant virus at week 12.

The first AZT-selected mutation emerging was the K70R, which was detectable at delivery in 5/50 women in proportions of 2%–28%. The shortest interval between the start of AZT prophylaxis and detection of the K70R mutation was 28 days (Table 3, no 3). T215Y and T215F mutations mostly emerged later and were measurable 1–6 weeks postpartum in 4/50 (8%) women in low proportions of 0.5%–3.9%. One woman displayed both AZT resistance mutations K70R and T215F in the viral genome, which were present already at delivery and persisted throughout the observation period at low frequencies (Table 3, no 5).

The total median viral load reduction from baseline to delivery was 0.6 $\log_{10}$; women with AZT-resistant virus at delivery displayed significantly lower reduction (0.1 $\log_{10}$) compared to women without AZT resistance at delivery ($p = 0.045$, Mann-Whitney U-test). Accordingly, women with AZT-resistant virus at delivery displayed significantly higher median viral load at delivery (29400 copies/ml) compared to women without AZT resistance at delivery (2680 copies/ml; $p = 0.021$, Mann-Whitney U-test). Furthermore, women exhibiting AZT-resistant virus at delivery had lower CD4 cell counts at baseline (331 cells/mm^3) versus women without AZT resistance (406 cells/mm^3); this difference marginally failed to reach statistical significance ($p = 0.077$, Mann-Whitney U-test).

Table 2. Accuracy, inter-assay variability and detection limit of ASPCR assays to detect drug-resistant HIV-1 variants calculated from 7–9 independent experiments.

Input mutant allele (%)	Measured mean mutant allele (% ± standard deviation)													
	K70R (AGA)		K103N (AAC)		K103N (AAT)		Y181C (TGT)		M184V (GTG)		T215Y (TAC)		T215F (TTC)	
100	110	±33.6	115	±48.9	102	±20.4	108	±23.7	112	±24.5	116	±40.9	115	±31.5
10.0	9.35	±2.74	10.2	±2.59	10.9	±3.94	9.28	±2.72	8.38	±1.02	9.17	±2.63	11.7	±5.40
1.00	1.11	±0.42	0.85	±0.23	1.07	±0.42	1.12	±0.39	1.11	±0.22	1.09	±0.46	1.01	±0.47
0.10	0.29	±0.08	0.12	±0.05	0.10	±0.03	0.30	±0.08	0.27	±0.03	0.12	±0.06	0.11	±0.06
0	0.19	±0.08	0.01	±0.01	0.01	±0.01	0.08	±0.06	0.23	±0.03	0.05	±0.03	0.09	±0.04
Detection limit (%)	0.99		0.04		0.01		0.35		0.63		0.33		0.42	

doi:10.1371/journal.pone.0032055.t002

Table 3. Drug-resistant HIV-1 variants in plasma samples of 20/50 women after complex antiretroviral prophylaxis as analyzed by allele-specific PCR (ASPCR).

No	Sub-type	Viral load delivery (cop/ml)	Antenatal AZT-intake (days)	Results of population sequencing and ASPCR							
				Delivery		Week 1–2		Weeks 4–6		Weeks 12–16	
				popseq	ASPCR	popseq	ASPCR	popseq	ASPCR	popseq	ASPCR
1	C	1,546	58	K70R	13% K70R		wt		-		wt
2	C	29,400	77	K70R	11% K70R	wt	0.7% M184V		wt		-
3	A1	97,450	28	K70R	14% K70R		wt	wt	5.4% K70R		-
4	A1	7,915	81	K70R	28% K70R		wt	K70R	14% K70R		wt
5	C	37,800	81	wt	2.0% K70R 0.5% T215F	K65R	0.5% T215F	wt	2.3% K70R	wt	0.7% T215F
6	A1	4,806	43		wt	wt	0.5%T215Y		wt		wt
7	A1	6,400	87		wt	wt	10% K103N	wt	0.8%Y181C		-
8	C	3,790	49		wt	wt	0.4% Y181C	wt	1.3% K103N		-
9	C	21,800	14		wt	wt	0.6% M184V	wt	3.4% K103N		wt
10	A1	3,455	95		wt		wt	wt	4.9% K70R		-
11	C	1,002	92		wt		wt	wt	2.7% K70R		-
12	C	1,079	33		wt		-	wt	0.8% T215F		wt
13	C	4,625	32		wt		wt	wt	3.9% T215Y		wt
14	A1	646	65		wt		wt	wt	2.1% K103N		-
15	C	2,150	67		wt		wt	wt	3.4% K103N		-
16	C	2,875	49		wt		wt	K103NY181CV106A	36% K103N 20% Y181C 0.6% M184V	K103N	12% K103N 4.0% K70R
17	D	1,480	48		wt		-		wt	wt	0.2% K103N
18	C	1,258	38		wt		-		wt	wt	0.4% Y181C
19	C	1,055	56		wt		wt		wt	G190A	1.5% Y181C
20	C	47,050	56		wt		wt		wt	wt	1.0% M184V

wt = wild-type HIV-1.
- = no sample/not amplifiable.
doi:10.1371/journal.pone.0032055.t003

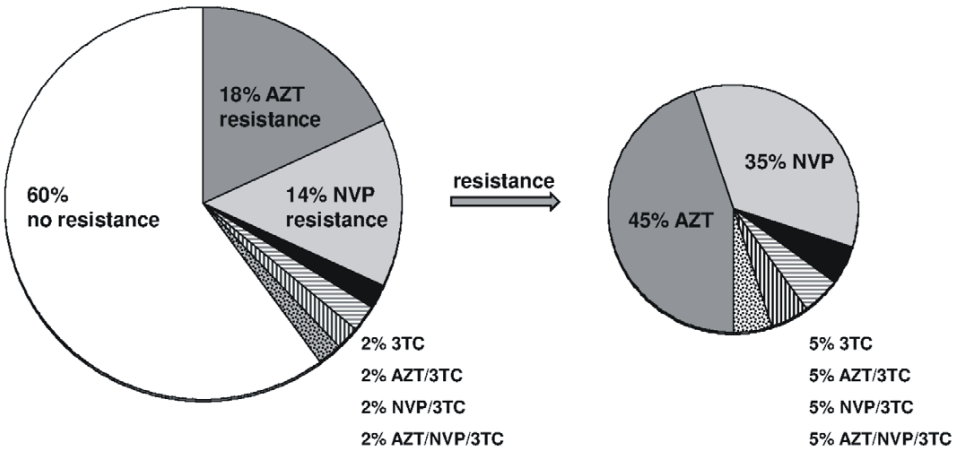

Figure 1. Distribution of drug-resistant HIV-1 variants after complex antiretroviral prophylaxis in 50 Tanzanian women.
doi:10.1371/journal.pone.0032055.g001

The median number of days of antenatal AZT intake did not differ significantly between the five women who displayed AZT resistance mutation at delivery (77 days) and the 45 women without AZT-resistance at delivery (50 days; p = 0.20, Mann-Whitney U-test). However, the frequency of AZT resistance at delivery differed significantly in women with antenatal AZT intake of at least 10 weeks (3/10 = 33%) as compared to women who took antenatal AZT for less than 10 weeks (2/40 = 5%; p = 0.048, Fisher's exact test).

NVP resistance mutations K103N and/or Y181C were detected in postpartum samples of nine (18%) women, but the proportion of resistant variants never exceeded 5% during the study period in 7/9 (78%) of these women. In 2/9 (22%) women higher proportions were detectable (Table 3, nos. 7, 16). One of these women (no. 16) did take NVP-SD and AZT/3TC during labor, but did not receive the postpartum AZT/3TC-tail to avoid NVP-resistance development. This woman exhibited dual-resistant virus against NVP and 3TC at week 4–6 and dual-resistant virus against NVP and AZT at month three. 3/9 women who had not taken AZT and/or 3TC postpatum (Table 3, nos. 16, 17, 19) developed NVP-resistance compared to 6/41 women who took the postpartal tail correctly (p = 0.33, Fisher's exact test).

The 3TC-resistance mutation M184V was detected in four women (8%) in low proportions of 0.6%–1.0% and was no longer detectable in 3/4 women at week 12–16.

In 70% (14/20) of the women who developed drug-resistant HIV-1 variants the relative proportions of resistant populations never exceeded 5% during the whole study period. The range of proportions of drug-resistant HIV-1 variants was 0.2–36% for K103N mutants, 0.4–20% for Y181C mutants, 0.6–1.0% for M184V mutants, 2.0–28% for K70R mutants, 0.5–3.9% for T215Y mutants and 0.5–0.8% for T215F mutants, respectively. In total, 34 drug-resistant variants were detected; out of these, 12 were present in proportions <1%, 12 in proportions of 1–5%, and 10 in proportions of >5%.

Altogether, complex prophylaxis resulted in the development of drug resistance in 40% of HIV-infected women. Out of these, 45% carried HIV-1 with AZT-resistance mutations, 35% showed NVP single drug-resistance, 5% 3TC single drug-resistance and 15% dual or triple drug-resistance in the viral genome (Figure 1). A longer duration of antenatal AZT intake seemed to increase the risk for selection of AZT-resistance mutations. In most women drug-resistant virus was present as minority species only.

Vertical transmission and emergence of drug-resistant HIV-1 variants in infected infants

Blood specimens collected 4–6 weeks after birth were available for 47/50 newborns; 5 were tested to be HIV-positive (no. 5, 6, 13, 21, 22; Table 4). In three additional cases, the 4–6 week sample was lacking, and an earlier sample (taken at delivery, 3 days or 2 weeks postpartum) was analyzed respectively: two of these samples were HIV-PCR positive, those infants were therefore assumend to be HIV-1 infected (no. 23, 24; Table 4). The third child was HIV-PCR negative, this infant was excluded from calculation of the transmission-rate. The overall HIV-transmission rate 4–6 weeks after birth was 14.3% (7/49 infants).

Vertical transmission was proven by phylogenetic analysis of maternal and infant HIV-1 sequences (data not shown). We did not observe a correlation between the vertical transmission risk of HIV-1 with either maternal CD4 cell count at enrolment, viral load at delivery or viral load reduction during pregnancy (p = 0.131; p = 0.388; p = 0.360, Mann-Whitney U-test) or with the presence of AZT-resistant HIV-1 variants (p = 0.546, Fisher's exact test). All children were at least exposed to maternal NVP-SD during delivery, and 44/50 (88%) infants took an additional dose of NVP postnatally. Eleven plasma samples of the seven HIV-infected infants were amplifiable in outer PCR and were available for subsequent ASPCR assays (Table 4). Three of 7 infants developed drug-resistant virus (Table 4, nos. 5, 21 and 22). Two infants (nos. 21 and 22) developed NVP-resistant HIV variants while both mothers exhibited wild-type virus only during the observation time. To one of these infants (no. 22) neither postnatal NVP nor AZT was administered, but the child developed high proportions of NVP-resistant virus at week 4–6. The third newborn (no. 5) carried resistant virus against AZT (K70R) and NVP (K103N) 4–6 weeks after birth; the mutation K70R was also detectable in the maternal delivery sample.

Results of population-based sequencing and comparison with ASPCR results

Population-based sequencing was conducted on all maternal and infant samples with drug resistance mutations as determined by ASPCR (n = 34, Table 3 and Table 4) and additionally on 27 samples without indication of drug-resistant virus in the ASPCR (data not shown).

In all samples harboring resistant virus in proportions >20% according to ASPCR assays, population-based sequencing confirmed the presence of drug-resistant virus, and the presence of mutations as identified by population-based sequencing was always detected in the ASPCR assays (Table 3). All samples without detectable drug-resistant HIV-1 or with drug-resistant variants in proportions < = 10% in the ASPCR were identified to contain HIV wild-type only by population sequencing (Table 3).

We also checked population sequences for additional AZT/3TC/NVP-selected resistance mutations like M41L, D67N, K70R, L210W, T215Y/F and K219QE for AZT, K65R for 3TC and L100I, K101P, V106A/M, V108I, Y188C/L/H and G190A for NVP. Additional mutations in the HIV-1 genome were detected in three women: One woman each harbored the V106A (together with K103N, Y181C and M184V), the K65R (together with T215F) and the G190A (together with Y181C) mutation, respectively (Table 3, nos. 5, 16, 19).

Discussion

Since 2006, WHO PMTCT guidelines recommend complex antiretroviral prophylaxis with AZT monotherapy during pregnancy, NVP-SD at labor onset, AZT/3TC during labor and for one week after delivery [14,15]. Since AZT monotherapy and usage of drugs with low genetic barriers like NVP and 3TC might facilitate the formation of drug resistance, we aimed at monitoring the emergence and persistence of key resistance mutations selected by AZT, NVP and 3TC in 50 Tanzanian women from enrolment (before start of prophylaxis) up to three months postpartum. To our knowledge, this is the first study analyzing drug-resistance including minority species in women who had taken the WHO recommended complex prophylaxis.

AZT resistance

Emergence of AZT-resistant virus after starting AZT monotherapy during pregnancy has been reported to be low with less than 3% occurrence [38,39]. Applying our highly sensitive ASPCR assays capable of detecting minority species <1%, we detected HIV-1 with AZT-resistance mutations in a much higher proportion of women (11/50 = 22%). However, population-based sequencing, detecting minor variants in proportions only above 20%, revealed AZT-resistance mutations (K70R) in HIV-1 of only 4 women (8%). Furthermore, the women in our study displayed

Table 4. Drug-resistant HIV-1 variants in plasma samples of seven children HIV-1 infected by vertical transmission as analyzed by allele-specific PCR (ASPCR).

No	Sub-type	Mother/ child	Maternal CD4 count (cells/μl)	Maternal viral load (cop/ml)	Ante-natalAZT (days)	Drug intake during labor	Drug intake postnatal	Results of ASPCR			
								delivery	week 1–2	week 4–6	week 12–16
5	C	mother	344	37,800	81	NVP-SD	AZT/3TC	2.0% K70R° 0.5% T215F°	0.5% T215F°	2.3% K70R°	0.7% T215F°
		child					NVP-SD AZT	-	-	15% K70R * 3.4% K103N°	2.7% K70R°
6	A1	mother	572	4,806	43	NVP-SD AZT/ 3TC	AZT/3TC	wt	0.5%T215Y°	wt	wt
		child					NVP-SD AZT	n/a	-	wt	n/a
13	C	mother	678	4,625	32	NVP-SD AZT/ 3TC	AZT/3TC	wt	wt	3.9% T215Y°	wt
		child					NVP-SD AZT	wt	-	wt	wt
21	A1	mother	231	14,850	33	NVP-SD AZT	AZT/3TC	wt	wt	wt	-
		child					NVP-SD AZT	-	-	0.9% K103N° 2.5% Y181C°	-
22	C	mother	211	1,720	60	NVP-SD	-	wt	n/a	wt	-
		child					-	-	-	12% K103N° 12% Y181C°	-
23	C	mother	612	2,110	20	NVP-SD AZT/ 3TC	AZT/3TC	wt	wt	wt	wt
		child					NVP-SD AZT	n/a	wt	-	wt
24	A1	mother	200	5,385	46	NVP-SD AZT/ 3TC	AZT/3TC	wt	wt	wt	-
		child					NVP-SD AZT	n/a	wt #	-	-

wt = wild-type HIV-1.
n/a = not amplifiable.
- = no sample.
= sample collected at day 3.
* = also detected by population-based sequencing.
° = not detected by population-based sequencing.
doi:10.1371/journal.pone.0032055.t004

lower CD4 cell count levels (median: 390 cells/mm^3) compared to the relatively immunocompetent women in other studies (median: >500 cells/mm^3) [38,39]. Advanced disease stage and low CD4 cell counts have been shown to be associated with a higher frequency of AZT-resistance [40,41]. This is in accordance with our finding, that women carrying virus variants with AZT-selected mutations at delivery displayed a 10fold higher median viral load compared to women without AZT resistance mutation at delivery ($p = 0.021$, Mann-Whitney U-test). Furthermore, these women tended to display lower CD4 cell counts (median: 331 cells/mm^3) in comparison to women without AZT resistance mutations (median: 406 cells/mm^3; $p = 0.077$, Mann-Whitney U-test). In the most recent WHO guidelines (2010), AZT prophylaxis is recommended to start at a higher CD4 cell count level of 350 cells/mm^3 instead of 200 cells/mm^3 as in the previous 2006 guidelines. This might contribute to reduced emergence of AZT resistant HIV-1.

The shortest interval between start of AZT exposure and the emergence of AZT-selected mutation K70R was 28 days only. AZT resistance mutations were detected more frequently in HIV-1 of women who had taken AZT during pregnancy for longer than 10 weeks. In fact, in 30% of these women HIV carried AZT-resistance mutations at delivery. It is well known from other studies that the duration of AZT intake is associated with resistance development [40,42,43].

K70R was the most frequently observed AZT mutation in samples taken at delivery ($n = 5$), while T215Y and T215F mutations mostly emerged later during the observation period. In fact, the K70R mutation is considered to be an early AZT mutation and indicates the emergence of AZT-resistance followed by M41L, T215Y/F and L210W [34]. This might be due to the fact that for K70R one base substitution is sufficient (AAA/AAG to AGA/AGG) while for T215Y and F two base mutations are required (ACC to TAC = >T215Y or TTC = >T215F) [34]. 7/11 women with HIV-1 carrying AZT-selected mutants displayed the K70R mutation in proportions of 3%–28%, whereas T215Y/F-carrying virus was harbored in lower proportions of 0.5%–3.9% by four women. It is important to note that the K70R mutation affecting HIV-1 of 7/50 (14%) women confers low level resistance towards AZT, whereas T215Y and T215F mutations affecting virus of 4/50 (8%) women result in high-level resistance [34,35]. While emergence of K70R is transient, AZT-resistant mutation T215Y is reported to persist for several months up to more than one year even after AZT discontinuation [44–46].

Antenatal AZT is supposed to reduce in-utero HIV-1 transmission. So far, it is not fully understood how exactly AZT is preventing in-utero transmission. Viral load reduction by AZT in pregnancy has been shown to be modest with -0.24 $\log_{10}$ and -0.3 $\log_{10}$ by Sperling [47] and Clarke [48] and with -0.6 $\log_{10}$

in our study. Therefore, since AZT readily crosses the placenta [49] it is rather conceivable that the child is at least also protected by pre- and post-exposure prophylaxis than by the maternal viral load reduction at delivery.

Since the AZT resistance mutation T215Y was shown to persist for several month [44–46], resistant variants could be re-selected if exposed to prophylactic AZT in future pregnancies or during subsequent AZT-containing HAART if initiated within this period after AZT exposure. This is of special importance for Sub-Saharan African populations as many women give birth to more than one child; AZT mutations may accumulate over time if AZT is used during consecutive pregnancies.

Our results are conflicting with the WHO statement that "the available evidence suggests that the time-limited use of AZT monotherapy during pregnancy for prophylaxis (for approximately six months, or less) should not be associated with a significant risk of developing AZT resistance" [15]. Compared to 2006, WHO guidelines from 2010 recommend to prepone the start of antenatal AZT to week 14 instead of week 28 [14,15], corresponding to a 6-month AZT monotherapy. According to our findings, prolongation of antenatal AZT may increase the frequency of AZT-resistant virus.

NVP and 3TC resistance

NVP-selected resistance mutations that cause cross-resistance to other NNRTIs are a major concern as NNRTIs are cornerstones of first-line HAART in resource-constrained settings. According to WHO guidelines, AZT/3TC should be taken by women for seven days postpartum to counteract the long presence of subtherapeutic NVP concentrations due to NVP's long half-life. NVP resistance was detected in 18% in our study group, which is a remarkable reduction compared to up to 87% after NVP-SD intervention [10]. The efficacy of postpartum short-course AZT/3TC-tails in reducing NNRTI resistance after intrapartum NVP-SD has indeed been shown in other studies [50,51]. In our study group, 8% of women exhibited 3TC-resistant virus in very low proportions of $<1\%$ only. The M184V mutation results in complete resistance to 3TC and the presence of postpartum M184V in proportions $>20\%$ has been correlated to subsequent treatment failure using 3TC-containing HAART [52]. However, the clinical and virological relevance of 3TC-resistant virus in low proportions is not known. Moreover, M184V is known to be rapidly lost upon withdrawal of 3TC.

Multiple drug resistance

In three women, resistant virus against more than one drug emerged during the observation period. The main risk factor for resistance development in general is incomplete adherence. The most severely affected woman with respect to HIV-1 resistance development (Table 3, no. 16) did not take AZT/3TC postpartum; it seems reasonable to assume that this fostered resistance development. It could be argued that the resistance development in this woman cannot be attributed to the effect of complex prophylaxis as it was not taken correctly. However, this might as well realistically reflect the existing conditions in rural settings and the challenges to adhere to a complex drug regimen.

Minor drug resistance

In 70% (14/20) of the women with development of drug-resistant HIV-1, the resistant variants never exceeded proportions of 5%. The clinical relevance of these minority species is not fully understood and controversially discussed [17–24,53]. There is evidence that minor drug-resistant variants can re-emerge in subsequent regimens leading to failure of salvage therapy [21]. While Metzner et al. [53] reported of successful treatment despite pre-existing minor K65R, K103N and M184V-variants in German Truvada cohort, several other studies have shown that the presence of drug-resistant minor variants increased the risk for subsequent treatment failure for NNRTI- [18–24], protease inhibitor- [17,54,55] and AZT-containing treatment [56]. While a single NNRTI-resistance mutation confers high-level resistance to some NNRTIs (an association with virologic failure in efavirenz-containing regimen was found for K103N variants at frequencies of $>=0.5\%$ by Halvas et al. [57]), resistance to PI and AZT requires an accumulation of several mutations [58]. It is not yet fully understood at which threshold minor resistant viral populations may become clinically relevant. Furthermore, the threshold might be different for each resistance mutation and also depend on the subsequent treatment regimen. More evidence-based data are necessary to determine the role of minor drug-resistant HIV-1 in the response to antiretroviral therapy.

Vertical transmission and emergence of drug-resistant HIV-1 variants in infected infants

The overall transmission rate in this study cohort of 50 mother-infant pairs 4–6 weeks after delivery was 14.3% and thus unexpectedly high. Neither a low CD4 cell count nor a high viral load at delivery in the transmitting mothers could be identified as transmission risk factors. Of 50 infants, all but one were breastfed, including all HIV-infected infants. We could not define the exact time of transmission for 4/7 infants due to lacking samples of delivery and/or of week 1–2. However, at least 3/7 children were born HIV uninfected (HIV-PCR was negative in the delivery sample). We therefore assume that postpartal transmission via breastmilk is the main reason for the high transmission rate.

Three of 7 infants developed drug-resistant HIV-1. In 2/3 newborns with NVP-resistant variants, mutations most likely emerged in the infants as both mothers exhibited wild-type HIV-1 only during the observation period. One infant, who did not take AZT and NVP postnatally (no. 22) exhibited NVP-resistant virus in high proportions at week 4–6 which was selected most likely by the maternal NVP dose. NVP rapidly crosses the placenta, resulting in high NVP concentrations in the infant's blood at birth [59,60]. Postnatal NVP dosing of the infant only slightly elevated the NVP levels in infants [61]. Therefore an infant whose mother has taken NVP-SD during labor can develop NVP-resistant virus even without postnatal ingestion of NVP.

Conclusions

Although complex antiretroviral prophylaxis decreased NVP-selected resistance compared to NVP-SD alone, HIV-1 with AZT-resistance mutations emerged in a substantial proportion of women. This may impact negatively future AZT-containing prophylaxis and HAART of the mother. In accordance with Katzenstein [62], we believe that it should be considered to substitute AZT monotherapy in pregnancy by HAART. There is growing evidence that starting HAART regardless of CD4 cell count level is highly beneficial for all HIV-infected individuals [63–66]. Additionally, HAART during pregnancy seems to be safe and advantageous for maternal and infant health [67–70] although it is important to further monitor the long-term effects of antiretroviral drugs on HIV-exposed but uninfected children [71]. In the light of the accumulating knowledge on the detrimental nature of untreated HIV-1, it seems justified to treat this infectious disease as soon as it is diagnosed instead of delaying medication until destructions of immune functions have taken place. Therefore, we advocate for HAART for *all* HIV-positive pregnant women; this equals "option B" in WHO guidelines of

2010 [15]. However, beyond that HAART should be considered lifelong and not be stopped after delivery, as discontinuation increases the risk of future treatment failure when restarting HAART [72]. This approach would minimize the risk of HIV-1 transmission and of resistance development, would allow breast-feeding and have an overall beneficial impact on HIV-1-infected mothers and their children.

Supporting Information

Materials and Methods S1
(DOC)

References

1. Joint United Nations Program on HIV/AIDS (UNAIDS) (2010) Global Report: UNAIDS report on the global AIDS epidemic 2010. Available: http://www.unaids.org/globalreport/Epi_slides.htm). Accessed 7 September 2011.
2. Guay LA, Musoke P, Fleming T, Bagenda D, Allen M, et al. (1999) Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomised trial. Lancet 354: 795–802.
3. Eshleman SH, Guay LA, Wang J, Mwatha A, Brown ER, et al. (2005) Distinct patterns of emergence and fading of K103N and Y181C in women with subtype A vs. D after single-dose nevirapine: HIVNET 012. J Acquir Immune Defic Syndr 40: 24–29.
4. Flys T, Nissley DV, Claasen CW, Jones D, Shi C, et al. (2005) Sensitive drug-resistance assays reveal long-term persistence of HIV-1 variants with the K103N nevirapine (NVP) resistance mutation in some women and infants after the administration of single-dose NVP: HIVNET 012. J Infect Dis 192: 24–29.
5. Johnson JA, Li JF, Morris L, Martinson N, Gray G, et al. (2005) Emergence of drug-resistant HIV-1 after intrapartum administration of single-dose nevirapine is substantially underestimated. J Infect Dis 192: 16–23.
6. Loubser S, Balfe P, Sherman G, Hammer S, Kuhn L, et al. (2006) Decay of K103N mutants in cellular DNA and plasma RNA after single-dose nevirapine to reduce mother-to-child HIV transmission. AIDS 20: 995–1002.
7. Palmer S, Boltz V, Martinson N, Maldarelli F, Gray G, et al. (2006) Persistence of nevirapine-resistant HIV-1 in women after single-dose nevirapine therapy for prevention of maternal-to-fetal HIV-1 transmission. Proc Natl Acad Sci U S A 103: 7094–7099.
8. Flys TS, Donnell D, Mwatha A, Nakabiito C, Musoke P, et al. (2007) Persistence of K103N-containing HIV-1 variants after single-dose nevirapine for prevention of HIV-1 mother-to-child transmission. J Infect Dis 195: 711–715.
9. Hauser A, Mugenyi K, Kabasinguzi R, Kuecherer C, Harms G, et al. (2011) Emergence and Persistence of Minor Drug-Resistant HIV-1 Variants in Ugandan Women after Nevirapine Single-Dose Prophylaxis. PLoS One 6: e20357.
10. Arrive E, Newell ML, Ekouevi DK, Chaix ML, Thiebaut R, et al. (2007) Prevalence of resistance to nevirapine in mothers and children after single-dose exposure to prevent vertical transmission of HIV-1: a meta-analysis. Int J Epidemiol 36: 1009–1021.
11. Jourdain G, Ngo-Giang-Huong N, Le Coeur S, Bowonwatanuwong C, Kantipong P, et al. (2004) Intrapartum exposure to nevirapine and subsequent maternal responses to nevirapine-based antiretroviral therapy. N Engl J Med 351: 229–240.
12. Lockman S, Shapiro RL, Smeaton LM, Wester C, Thior I, et al. (2007) Response to antiretroviral therapy after a single, peripartum dose of nevirapine. N Engl J Med 356: 135–147.
13. Stringer JSA, McConnell MS, Kiarie J, Bolu O, Anekthananon T, et al. (2010) Effectiveness of Non-nucleoside Reverse-Transcriptase Inhibitor-Based Antiretroviral Therapy in Women Previously Exposed to a Single Intrapartum Dose of Nevirapine: A Multi-country, Prospective Cohort Study. PLoS Med 7: e1000233.
14. World Health Organization (WHO) (2006) Antiretroviral drugs for treating pregnant women and preventing HIV infection in infants: Towards universal access: Recommendations for a public health approach. 2006 version. Available: http://www.who.int/hiv/pub/guidelines/pmtctguidelines3.pdf Accessed 7 September 2011.
15. World Health Organization (WHO) (2010) Antiretroviral drugs for treating pregnant women and preventing HIV infection in infants: Recommendations for a public health approach. 2010 version. Available: http://whqlibdoc.who.int/publications/2010/9789241599818_eng.pdf. Accessed 7 September 2011.
16. The United Republic of Tanzania, Ministry of Health and Social Welfare (2007) Prevention of Mother-To-Child Transmission of HIV (PMTCT), National Guidelines. Dar es Salaam, Tanzania: Ministry of Health and Social Welfare, Available at: http://www.nacp.go.tz/documents/PMTCT%20GUIDELINES%202007.pdf. Accessed 3 August 2011.
17. Charpentier C, Dwyer DE, Mammano F, Lecossier D, Clavel F, et al. (2004) Role of minority populations of human immunodeficiency virus type 1 in the evolution of viral resistance to protease inhibitors. J Virol 78: 4234–4247.
18. Lecossier D, Shulman NS, Morand-Joubert L, Shafer RW, Joly V, et al. (2005) Detection of minority populations of HIV-1 expressing the K103N resistance mutation in patients failing nevirapine. J Acquir Immune Defic Syndr 38: 37–42.
19. Johnson JA, Li JF, Wei X, Lipscomb J, Irlbeck D, et al. (2008) Minority HIV-1 drug resistance mutations are present in antiretroviral treatment-naive populations and associate with reduced treatment efficacy. PLoS Med 5: e158.
20. Coovadia A, Hunt G, Abrams EJ, Sherman G, Meyers T, et al. (2009) Persistent minority K103N mutations among women exposed to single-dose nevirapine and virologic response to nonnucleoside reverse-transcriptase inhibitor-based therapy. Clin Infect Dis 48: 462–472.
21. Metzner KJ, Giulieri SG, Knoepfel SA, Rauch P, Burgisser P, et al. (2009) Minority quasispecies of drug-resistant HIV-1 that lead to early therapy failure in treatment-naive and -adherent patients. Clin Infect Dis 48: 239–247.
22. MacLeod IJ, Rowley CF, Thior I, Wester C, Makhema J, et al. (2010) Minor resistant variants in nevirapine-exposed infants may predict virologic failure on nevirapine-containing ART. J Clin Virol 48: 162–167.
23. Rowley CF, Boutwell CL, Lee EJ, MacLeod IJ, Ribaudo HJ, et al. (2010) Ultrasensitive detection of minor drug-resistant variants for HIV after nevirapine exposure using allele-specific PCR: clinical significance. AIDS Res Hum Retroviruses 26: 293–300.
24. Li JZ, Paredes R, Ribaudo HJ, Svarovskaia ES, Metzner KJ, et al. (2011) Low-frequency HIV-1 drug resistance mutations and risk of NNRTI-based antiretroviral treatment failure: a systematic review and pooled analysis. JAMA 305: 1327–1335.
25. Johnson VA, Brun-Vezinet F, Clotet B, Gunthard HF, Kuritzkes DR, et al. (2010) Update of the drug resistance mutations in HIV-1: December 2010. Top HIV Med 18: 156–163.
26. HIV Drug Resistance Database. Mutation Prevalence According to Subtype and Treatment. Available: http://hivdb.stanford.edu/cgi-bin/MutPrevBySubtypeRx.cgi. Accessed 7 September 2011.
27. Arroyo MA, Hoelscher M, Sateren W, Samky E, Maboko L, et al. (2005) HIV-1 diversity and prevalence differ between urban and rural areas in the Mbeya region of Tanzania. AIDS 19: 1517–1524.
28. Kirsten I, Sewangi J, Kunz A, Dugange F, Ziske J, et al. (2011) Adherence to Combination Prophylaxis for Prevention of Mother-to-Child-Transmission of HIV in Tanzania. PLoS One 6: e21020.
29. Hauser A, Mugenyi K, Kabasinguzi R, Bluethgen K, Kuecherer C, et al. (2009) Detection and quantification of minor human immunodeficiency virus type 1 variants harboring K103N and Y181C resistance mutations in subtype A and D isolates by allele-specific real-time PCR. Antimicrob Agents Chemother 53: 2965–2973.
30. Metzner KJ, Rauch P, Walter H, Boesecke C, Zollner B, et al. (2005) Detection of minor populations of drug-resistant HIV-1 in acute seroconverters. AIDS 19: 1819–1825.
31. Halvas EK, Aldrovandi GM, Balfe P, Beck IA, Boltz VF, et al. (2006) Blinded, multicenter comparison of methods to detect a drug-resistant mutant of human immunodeficiency virus type 1 at low frequency. J Clin Microbiol 44: 2612–2614.
32. Paredes R, Marconi VC, Campbell TB, Kuritzkes DR (2007) Systematic evaluation of allele-specific real-time PCR for the detection of minor HIV-1 variants with pol and env resistance mutations. J Virol Methods 146: 136–146.
33. Rowley CF, Boutwell CL, Lockman S, Essex M (2008) Improvement in allele-specific PCR assay with the use of polymorphism-specific primers for the analysis of minor variant drug resistance in HIV-1 subtype C. J Virol Methods 149: 69–75.
34. Boucher CA, O'Sullivan E, Mulder JW, Ramautarsing C, Kellam P, et al. (1992) Ordered appearance of zidovudine resistance mutations during treatment of 18 human immunodeficiency virus-positive subjects. J Infect Dis 165: 105–110.
35. HIV Drug Resistance Database. NRTI Resistance Notes. Available: http://hivdb.stanford.edu/cgi-bin/NRTIResiNote.cgi. Accessed 7 September 2011.
36. Bioedit. Version 7.0.5. Available: http://www.mbio.ncsu.edu/BioEdit/bioedit.html. Accessed 7 September 2011.
37. de Oliveira T, Deforche K, Cassol S, Salminen M, Paraskevis D, et al. (2005) An automated genotyping system for analysis of HIV-1 and other microbial sequences. Bioinformatics 21: 3797–3800.

Acknowledgments

We sincerely thank all women who participated in this study with their children. We are grateful to the staff of Kyela District Hospital who made this study possible.

Author Contributions

Analyzed the data: AH IL JZ CK AK. Contributed reagents/materials/analysis tools: CK GH. Wrote the paper: AH JS PM FD IL JZ ST CK GH AK. Designed the experiments/the study: AH IL CK GH AK. Collected data/did experiments for the study: AH JS PM FD IL JZ ST AK. Enrolled patients: JS PM FD IL JZ.

38. Eastman PS, Shapiro DE, Coombs RW, Frenkel LM, McSherry GD, et al. (1998) Maternal viral genotypic zidovudine resistance and infrequent failure of zidovudine therapy to prevent perinatal transmission of human immunodeficiency virus type 1 in pediatric AIDS Clinical Trials Group Protocol 076. J Infect Dis 177: 557–564.
39. Ekpini RA, Nkengasong JN, Sibailly T, Maurice C, Adjé C, et al. (2002) Changes in plasma HIV-1-RNA viral load and CD4 cell counts, and lack of zidovudine resistance among pregnant women receiving short-course zidovudine. AIDS 16: 625–630.
40. Land S, McGavin C, Lucas R, Birch C (1992) Incidence of zidovudine-resistant human immunodeficiency virus isolated from patients before, during, and after therapy. J Infect Dis 166: 1139–1142.
41. Richman DD, Grimes JM, Lagakos SW (1990) Effect of stage of disease and drug dose on zidovudine susceptibilities of isolates of human immunodeficiency virus. J Acquir Immune Defic Syndr 3: 743–746.
42. Nielsen C, Gøtzsche PC, Nielsen CM, Gerstoft J, Vestergaard BF (1992) Development of resistance to zidovudine in HIV strains isolated from CD4+ lymphocytes and plasma during therapy. Antiviral Res 18: 303–316.
43. Welles SL, Pitt J, Colgrove R, McIntosh K, Chung PH, et al. (2000) HIV-1 genotypic zidovudine drug resistance and the risk of maternal–infant transmission in the women and infants transmission study. The Women and Infants Transmission Study Group. AIDS 14: 263–271.
44. Albert J, Wahlberg J, Lundeberg J, Cox S, Sandstrom E, et al. (1992) Persistence of azidothymidine-resistant human immunodeficiency virus type 1 RNA genotypes in posttreatment sera. J Virol 66: 5627–5630.
45. Boucher CA, van Leeuwen R, Kellam P, Schipper P, Tijnagel J, et al. (1993) Effects of discontinuation of zidovudine treatment on zidovudine sensitivity of human immunodeficiency virus type 1 isolates. Antimicrob Agents Chemother 37: 1525–1530.
46. Smith MS, Koerber KL, Pagano JS (1994) Long-term persistence of AZT-resistance mutations in the plasma HIV-1 of patients removed from AZT therapy. Leukemia 8 Suppl 1: S179–182.
47. Sperling RS, Shapiro DE, Coombs RW, Todd JA, Herman SA, et al. (1996) Maternal viral load, zidovudine treatment, and the risk of transmission of human immunodeficiency virus type 1 from mother to infant. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. N Engl J Med 335: 1621–1629.
48. Clarke JR, Braganza R, Mirza A, Stainsby C, Ait-Khaled M, et al. (1999) Rapid development of genotypic resistance to lamivudine when combined with zidovudine in pregnancy. J Med Virol 59: 364–368.
49. Schenker S, Johnson RF, King TS, Schenken RS, Henderson GI (1990) Azidothymidine (zidovudine) transport by the human placenta. Am J Med Sci 299: 16–20.
50. Farr SL, Nelson JA, Ng'ombe TJ, Kourtis AP, Chasela C, et al. (2010) Addition of 7 days of zidovudine plus lamivudine to peripartum single-dose nevirapine effectively reduces nevirapine resistance postpartum in HIV-infected mothers in Malawi. J Acquir Immune Defic Syndr 54: 515–523.
51. McIntyre JA, Hopley M, Moodley D, Eklund M, Gray GE, et al. (2009) Efficacy of short-course AZT plus 3TC to reduce nevirapine resistance in the prevention of mother-to-child HIV transmission: a randomized clinical trial. PLoS Med 6: e1000172.
52. Coffie PA, Ekouevi DK, Chaix ML, Tonwe-Gold B, Clarisse AB, et al. (2008) Maternal 12-month response to antiretroviral therapy following prevention of mother-to-child transmission of HIV type 1, Ivory Coast, 2003–2006. Clin Infect Dis 46: 611–621.
53. Metzner KJ, Rauch P, Braun P, Knechten H, Ehret R, et al. (2011) Prevalence of key resistance mutations K65R, K103N, and M184V as minority HIV-1 variants in chronically HIV-1 infected, treatment-naïve patients. J Clin Virol 50: 156–61.
54. Dykes C, Najjar J, Bosch RJ, Wantman M, Furtado M, et al. (2004) Detection of drug-resistant minority variants of HIV-1 during virologic failure of indinavir, lamivudine, and zidovudine. J Infect Dis 189: 1091–6.
55. Roquebert B, Malet I, Wirden M, Tubiana R, Valantin MA, et al. (2006) Role of HIV-1 minority populations on resistance mutational pattern evolution and susceptibility to protease inhibitors. AIDS 20: 287–9.
56. Ross LL, Rouse E, Gerondelis P, DeJesus E, Cohen C, et al. (2010) Low-abundance HIV species and their impact on mutational profiles in patients with virological failure on once-daily abacavir/lamivudine/zidovudine and tenofovir. J Antimicrob Chemother 65: 307–15.
57. Halvas EK, Wiegand A, Boltz VF, Kearney M, Nissley D, et al. (2010) Low frequency nonnucleoside reverse-transcriptase inhibitor-resistant variants contribute to failure of efavirenz-containing regimens in treatment- experienced patients. J Infect Dis 201: 672–80.
58. Gianella S, Richman DD (2010) Minority variants of drug-resistant HIV. J Infect Dis 202: 657–66.
59. Mirochnick M, Fenton T, Gagnier P, Pav J, Gwynne M, et al. (1998) Pediatric AIDS Clinical Trials Group Protocol 250 Team. Pharmacokinetics of nevirapine in human immunodeficiency virus type 1-infected pregnant women and their neonates. J Infect Dis 178: 368–74.
60. Kunz A, Frank M, Mugenyi K, Kabasinguzi R, Weidenhammer A, et al. (2009) Persistence of nevirapine in breast milk and plasma of mothers and their children after single-dose administration. J Antimicrob Chemother 63: 170–7.
61. Frank M, von Kleist M, Kunz A, Harms G, Schütte C, et al. (2011) Quantifying the Impact of Nevirapine-Based Prophylaxis Strategies To Prevent Mother-to-Child Transmission of HIV-1: a Combined Pharmacokinetic, Pharmacodynamic, and Viral Dynamic Analysis To Predict Clinical Outcomes. Antimicrob Agents Chemother 55: 5529–40.
62. Katzenstein TL, Gerstoft J (2008) Zidovudine monotherapy in pregnancy: is it state of the art? HIV Med 9: 445–447.
63. Phillips AN, Gazzard B, Gilson R, Easterbrook P, Johnson M, et al. (2007) Rate of AIDS diseases or death in HIV-infected antiretroviral therapy-naive individuals with high CD4 cell count. AIDS 21: 1717–1721.
64. Ellis RJ, Badiee J, Vaida F, Letendre S, Heaton RK, et al. (2011) Nadir CD4 is a Predictor of HIV Neurocognitive Impairment in the Era of Combination Antiretroviral Therapy. AIDS 25: 1747–1751.
65. Kitahata MM, Gange SJ, Abraham AG, Merriman B, Saag MS, et al. (2009) Effect of early versus deferred antiretroviral therapy for HIV on survival. N Engl J Med 360: 1815–1826.
66. HIV-CAUSAL Collaboration (2010) The effect of combined antiretroviral therapy on the overall mortality of HIV-infected individuals. AIDS 24: 123–137.
67. Kesho Bora Study Group (2011) Triple antiretroviral compared with zidovudine and single-dose nevirapine prophylaxis during pregnancy and breastfeeding for prevention of mother-to-child transmission of HIV-1 (Kesho Bora study): a randomised controlled trial. Lancet Infect Dis 11: 171–180.
68. Marazzi MC, Liotta G, Nielsen-Saines K, Haswell J, Magid NA, et al. (2010) Extended antenatal antiretroviral use correlates with improved infant outcomes throughout the first year of life. AIDS 24: 2819–2826.
69. Marazzi MC, Palombi L, Nielsen-Saines K, Haswell J, Zimba I, et al. (2011) Extended antenatal use of triple antiretroviral therapy for prevention of HIV-1 mother-to-child transmission correlates with favourable pregnancy outcomes. AIDS 24: 1611–168.
70. Shapiro RL, Hughes MD, Ogwu A, Kitch D, Lockman S, et al. (2010) Antiretroviral regimens in pregnancy and breast-feeding in Botswana. N Engl J Med 362: 2282–2294.
71. Heidari S, Mofenson L, Cotton MF, Marlink R, Cahn P, et al. (2011) Antiretroviral Drugs for Preventing Mother-to-Child Transmission of HIV: A Review of Potential Effects on HIV-Exposed but Uninfected Children. J Acquir Immune Defic Syndr 57: 290–296.
72. Fox Z, Phillips A, Cohen C, Neuhaus J, Baxter J, et al. (2008) Viral resuppression and detection of drug resistance following interruption of a suppressive non-nucleoside reverse transcriptase inhibitor-based regimen. AIDS 22: 2279–2289.

Druckexemplar Publikation 3

Ziske J, Kunz A, Sewangi J, Lau I, Dugange F, Hauser A, Kirschner W, Harms G, Theuring S

Hematological changes in women and infants exposed to AZT-containing regimen for prevention of mother-to-child-transmission on HIV in Tanzania

PLoS ONE 2013;8(2):e55633

doi:10.1371/journal.pone.0055633

OPEN ACCESS Freely available online

PLOS ONE

Hematological Changes in Women and Infants Exposed to an AZT-Containing Regimen for Prevention of Mother-to-Child-Transmission of HIV in Tanzania

Judith Ziske[1], Andrea Kunz[1], Julius Sewangi[2], Inga Lau[1], Festo Dugange[3], Andrea Hauser[1], Wolf Kirschner[4], Gundel Harms[1], Stefanie Theuring[1]*

1 Institute of Tropical Medicine and International Health, Charité-Universitätsmedizin Berlin, Germany, **2** Regional AIDS Control Program Mbeya Region, Ministry of Health and Social Welfare, Mbeya, Tanzania, **3** Kyela District Hospital, Ministry of Health and Social Welfare, Kyela, Tanzania, **4** Forschung Beratung+Evaluation, Berlin, Germany

Abstract

Introduction: Tanzanian guidelines for prevention of mother-to-child-transmission of HIV (PMTCT) recommend an antiretroviral combination regimen involving zidovudine (AZT) during pregnancy, single-dosed nevirapine at labor onset, AZT plus Lamivudine (3TC) during delivery, and AZT/3TC for 1–4 weeks postpartum. As drug toxicities are a relevant concern, we assessed hematological alterations in AZT-exposed women and their infants.

Methods and Materials: A cohort of HIV-positive women, either with AZT intake (n = 82, group 1) or without AZT intake (n = 62, group 2) for PMTCT during pregnancy, was established at Kyela District Hospital, Tanzania. The cohort also included the infants of group 1 with an *in-utero* AZT exposure ≥4 weeks, receiving AZT for 1 week postpartum (n = 41), and infants of group 2 without *in-utero* AZT exposure, receiving a prolonged 4-week AZT tail (n = 58). Complete blood counts were evaluated during pregnancy, birth, weeks 4–6 and 12.

Results: For women of group 1 with antenatal AZT intake, we found a statistically significant decrease in hemoglobin level, red blood cells, white blood cells, granulocytes, as well as an increase in red cell distribution width and platelet count. At delivery, the median red blood cell count was significantly lower and the median platelet count was significantly higher in women of group 1 compared to group 2. At birth, infants from group 1 showed a lower median hemoglobin level and granulocyte count and a higher frequency of anemia and granulocytopenia. At 4–6 weeks postpartum, the mean neutrophil granulocyte count was significantly lower and neutropenia was significantly more frequent in infants of group 2.

Conclusions: AZT exposure during pregnancy as well as after birth resulted in significant hematological alterations for women and their newborns, although these changes were mostly mild and transient in nature. Research involving larger cohorts is needed to further analyze the impact of AZT-containing regimens on maternal and infant health.

Citation: Ziske J, Kunz A, Sewangi J, Lau I, Dugange F, et al. (2013) Hematological Changes in Women and Infants Exposed to an AZT-Containing Regimen for Prevention of Mother-to-Child-Transmission of HIV in Tanzania. PLoS ONE 8(2): e55633. doi:10.1371/journal.pone.0055633

Editor: Landon Myer, University of Cape Town, South Africa

Received April 23, 2012; **Accepted** December 29, 2012; **Published** February 6, 2013

Funding: The study was supported by the German Ministry of Economic Cooperation through the GIZ Sector Project ''Strengthening the German Contribution to the Global AIDS Response''. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: Author W. Kirschner is employed by "Forschung Beratung+Evaluation Berlin". They were involved for statistical counseling for data analysis. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

* E-mail: stefanie.theuring@charite.de

Introduction

Mother-to-child transmission of HIV has become a relatively rare event in most resource-rich countries, where vertical transmission nowadays occurs in less than 2% of cases [1]. This decline is based on a combination of several strategies, including early maternal diagnosis through routine counseling and HIV testing during antenatal care (ANC), provision of antiretroviral therapy (ART) or of antiretroviral (ARV) prophylaxis, elective Caesarean section and the complete avoidance of breastfeeding. The high requirements for this complex range of measures, such as access for women to a health care system, broad coverage of HIV testing among pregnant women, CD4 cell count monitoring, or affordable and sustainable replacement feeding [2], make it difficult to successfully reduce mother to child transmission of HIV (PMTCT) in resource-limited countries. Indeed, in 2010, ARV coverage for PMTCT was only about 50% in sub-Saharan Africa [3]. The implementation of a single-dose (sd) administration of the non-nucleoside reverse transcriptase inhibitor nevirapine (NVP) to mothers and babies in resource-poor countries has been a considerable step forward in PMTCT. However, although representing a simple, feasible and cost-effective regimen [4], a major problem of sdNVP is the high risk of inducing drug-resistant HIV variants. It has been shown that the addition of nucleoside reverse transcriptase inhibitors, such as Zidovudine (AZT) and Lamivudine (3TC), can significantly reduce this risk [5]. Furthermore, combining several drugs is more effective in reducing HIV-transmission and can result in transmission rates as low as 6.5% at six weeks postpartum [6].

Since 2006, the World Health Organization (WHO) PMTCT guidelines for resource-poor settings follow those findings and recommend a sequential combination prophylaxis, including antenatal AZT intake, sdNVP during labor and intra/postpartum AZT/3TC. Despite the clear advantages of this regimen in terms of efficacy and reduction in NVP resistance, it has nevertheless several drawbacks. As drug intake is supposed to last from pregnancy until the postpartum period, requiring different drugs at specific points in time, this prolonged and complex process can make adherence difficult [2,7]. Another important issue is that a combination prophylactic regimen obviously results into a much higher drug burden for mothers and infants than the previously recommended regimen. Previous retro- and prospective studies have shown that AZT interferes with hematopoiesis, resulting in decreased levels for several cell lineages in pregnant women [8,9]. Other studies have shown an impact on hematopoiesis with varying persistence in infants exposed to AZT *in utero* or postnatally [10,11,12,13]. Connor *et al.* [12] and Sperling *et al.* [14] found a transiently lower hemoglobin level that resolved within the first six to 12 weeks of age. Regarding other cell lineages, especially granulocytes, Le Chenadec *et al.* found that decreased levels persisted up to the age of 18 months [11]. As granulocytes are crucial for the immune response, with deficiency often leading to severe bacterial infections, and anemia can have life-threatening potential, monitoring is a crucial aspect, particularly in settings where treatment options are limited.

The United Republic of Tanzania, one of the poorest countries in the world [15], is also one of the countries most affected by the global HIV/AIDS epidemic. The general HIV prevalence is estimated to be 6%, while the prevalence of HIV in pregnant women is estimated to be 10% in major urban areas and 6% in less densely populated regions [16]. In 2008, Tanzania changed its PMTCT standard recommendation from sdNVP to a combination regimen in accordance with the 2006 WHO guidelines.

The aim of this study was to assess the potential hematological toxicity of the combination PMTCT regimen in women and infants in a peripheral setting in Tanzania.

Methods

Ethics Statement

The study was approved by the Tanzanian National Institute of Medical Research, by the Mbeya Region Ethical Committee, and by the Ethical Commission of Charité-Universitätsmedizin Berlin. Written informed consent was obtained from all participants, and all data remained confidential.

Setting, Procedures and Recruitment

An observational prospective follow-up study was conducted from September 2008 until September 2009 at the Kyela District Hospital (KDH) in Mbeya Region, Tanzania to accompany the introduction of ARV combination prophylaxis for MTCT with regard to feasibility and adherence [7]. Within the larger frame of this research, we performed a sub-study assessing hematological alterations linked to this regimen in mothers and infants.

Mbeya is among the regions in Tanzania with the highest HIV prevalence, estimated at around 9% of the general population [17]. KDH is a rural health facility in the south of Mbeya Region. Each month, approximately 130 pregnant women attend the ANC services of KDH for the first time. This hospital has been providing PMTCT services supported by the German Agency for International Cooperation since 2001. Combination ARV prophylaxis was introduced in KDH in March 2008 following the updated 2008 Tanzanian Guidelines.

Routine PMTCT procedures at KDH include voluntary HIV counseling and testing for all new antenatal care (ANC) clients visiting KDH. CD4 cell counts were performed for pregnant women identified as HIV-positive to assess their eligibility for either ART or ARV prophylaxis. Women with CD4 cell counts above 200 cells/mm^3 (and therefore not requiring ART for their own health) were eligible for ARV prophylaxis. Administration of AZT was initiated at week 28 of gestation or anytime thereafter. During delivery, the women received sdNVP, AZT and 3TC and were given a take-home postpartum tail of AZT for seven days. Women first identified as HIV-positive at the time of delivery, who therefore had no previous ARV intake, were also offered intra/postpartum ARV prophylaxis. Infants received sdNVP within 72 hours after birth and AZT syrup for one week if the mother had taken AZT for at least four weeks during pregnancy and for four weeks if the mother had taken AZT for less than four weeks.

The cohort included HIV-positive pregnant ART-naïve women aged 18 years or older who attended the ANC clinic and/or delivered in the maternity ward at KDH during the study period, who were eligible for ARV prophylaxis according to the guidelines and who had given informed consent. Women enrolled in ANC with pre-delivery AZT intake were assigned to group 1, and those who had no ARV prophylaxis during pregnancy but received drugs during delivery at the KDH maternity ward were assigned to group 2.

Group 1-women were included for the analysis of hematological changes during pregnancy. Infants of women participating in the study were eligible for analysis if they had received prophylaxis according to the guidelines, i.e. group 1-infants with postnatal AZT intake of one week and group 2-infants with four weeks (see graph 1). In accordance with the WHO guidelines for postnatal AZT prophylaxis in infants, the analysis of adverse effects in group 1-infants only included those with at least four weeks *in utero* exposure.

Samples and Data Collection

Data on the socio-demographic and clinical background of the women were collected through standardized, structured questionnaires for the stages of ANC, delivery and postpartum follow-up. The questionnaires were developed and pretested by the authors. Self-observed adverse events, adherence to ARV prophylaxis, and concomitant drug use were recorded at regular intervals throughout pregnancy (group 1), delivery (group 1 and 2) and during the postpartum period (groups 1 and 2). Maternal blood samples from group 1 were taken weekly in the first month of AZT intake, monthly throughout pregnancy, once at delivery, and at months one and three post-delivery. Blood was drawn from group 2 participants at delivery and again monthly during the first three months postpartum. Laboratory analysis included full blood counts for all samples, and CD4 cell counts were conducted with samples taken during the first ANC visit (group 1) and at delivery (groups 1 and 2). The comparability of the groups was confirmed with regard to the socio-demographic situation, CD4 cell count as well as malarial symptoms and malarial prophylaxis.

For the infants, cord blood was taken at delivery and further blood samples were taken during follow-up visits at one and three months of age. Full blood cell counts were performed for both mothers and infants. HIV-PCR was performed with samples from newborns taken at delivery and at one month of age and HIV-infected infants were excluded from the analysis as HIV infection itself causes hematological changes.

Sample taking and data collection in questionnaires and forms was performed by staff within the respective wards at KDH; a trained study nurse supervised this process.

Statistical Analysis

Data was analyzed using the statistical software Stata version 11. Descriptive analysis of maternal baseline information was performed to characterize the study population. Baseline characteristics of both groups were compared using the student's t-test, Mann-Whitney-U-test or Fisher's exact test.

Fixed effects models were used to follow the development of maternal hematological values during AZT intake, taking into account correlation within the woman herself and the variability between women. Coefficients are expressed as an alteration of parameter per day if the linear model was significant. Student's t-test for independent variables was used to compare hematological values of women by group at birth and to compare hematological parameters of infants at birth and one month of age. For data at three months of age, Mann-Whitney-U-test was performed, taking the dropout rate into account. To classify ARV induced toxicities, tables from the Division of AIDS (DAIDS) were used for grading the severity of adverse events in adults and infants [18]. At birth, hemoglobin levels between 10 g/dl and 8.5 g/dl were defined as mild anemia (grade 1); 8.4 g/dl to 7.5 g/dl as moderate anemia (grade 2); 7.4 g/dl to 6.5 g/dl as severe anemia (grade 3) and hemoglobin levels less than 7.4 g/dl as potentially life threatening anemia (grade 4). Granulocyte counts between 5/nl and 4/nl were defined as grade 1 toxicity; less than 4/nl to 3/nl as grade 2 toxicity; less than 3/nl to 1.5/nl as grade 3 toxicity and counts less than 1.5/nl as grade 4 toxicity. For follow-up visits, toxicity thresholds for granulocyte counts and hemoglobin levels were adjusted to age according to the DAIDS tables. Frequencies of adverse events were compared using Fisher's exact test. Prematurity was defined as delivery before 37 weeks of gestation. The Kaplan-Meier approach was used to estimate the cumulative proportion of infected infants at six weeks of age, representing short term efficacy [19]. As recommended by the Ghent Group [19,20], any PCR result collected from infants between 29 and 60 days of age in combination with earlier results were used for this estimation. A p-value below 0.05 was considered statistically significant.

Results

Study Population

During the study period, 1395 pregnant women were counseled and tested for HIV infection at the ANC clinic of KDH [7]. HIV infection was diagnosed in 220 women (15.8% of all tested), of whom 121 met the eligibility criteria for this study. The study population during all stages of observation is explained in Figure 1.

Eighty-two women had a pre-delivery AZT intake of at least one week and were therefore assigned to group 1. The median CD4 cell count at enrolment in this group was 390 (inter-quartile range [IQR]: 267 to 515) cells/mm^3 and the median gestational age at start of AZT intake was 29.1 (IQR: 28.0 to 32.2) weeks. Hematological parameters and the specifications of adverse events of these 82 women were analyzed during pregnancy. Fifty-five women of group 1 delivered at KDH with 41 infants born within this group having been exposed to AZT *in utero* for at least four weeks and identified as HIV-negative at birth. The median duration of AZT exposure was 57 (IQR: 43 to 71) days during pregnancy and the median CD4 cell count in mothers at delivery in this group was 350 (IQR: 252 to 490) cells/mm^3. Thirty-nine of the 41 mother-infant pairs returned for follow-up after one month. Two of the 39 infants were tested HIV positive at that point and were excluded from the analysis. At one month of age, 37 infants remained for assessing hematological alterations. Sixteen group 1-infants were available for blood analysis upon their three month return visit.

In the same period, 62 women meeting the inclusion criteria were enrolled at the time of delivery and assigned to group 2. The median CD4 cell count in these women was 262 (IQR: 194 to 474) cells/mm^3. Four infants of this group were identified as HIV-positive at delivery and were excluded from the cohort, leaving 58 infants available for the analysis at the time of birth. Twenty-three HIV-negative infants remained for analysis at one month of age, and 12 were returned for follow-up at three months.

Baseline characteristics of both groups, including age, weight, years of education, marital status and malarial symptoms did not differ significantly among the two groups (tables 1 and 2).

Hematological Alterations in Women

Eighty-two women in group 1 had an AZT intake of at least one week and were included in the analyses of blood alterations. Coefficients for alterations in blood components over time were estimated with a fixed effects model for the duration of AZT intake. Mean corpuscular volume (MCV) (coef.: 0.0636fl per day, $p<0.001$), red distribution width (RDW) (coef.: 0.0655% per day, $p<0.001$) and platelet count (coef.: 1.0309/nl per day, $p<0.001$) increased significantly over the time of AZT intake. Red blood count (RBC) (coef.: $-0.0040*10^6$/µl per day, $p<0.001$), white blood cell count (WBC) (coef.: −0.0119/nl per day, $p<0.001$) and granulocyte count (coef.: −0.0123/nl per day, $p<0.001$) decreased significantly over time. A significant decrease with a subsequent increase after day 32 of AZT intake was shown for hemoglobin values ($p<0.05$). Comparing group 1 and 2 at the point of delivery, differences between median hemoglobin and median granulocyte count were not statistically significant. Median red blood cell count was significantly lower in women of group 1 (3.66/µl vs. 4.04/µl, $p<0.05$), whereas MCV (87fl vs. 79fl, $p<0.001$) and median platelet count (367000/mm^3 vs. 273500/mm^3, $p<0.05$) were significantly higher in group 1-women with antenatal AZT intake. No significant differences in hematological parameters were observed at one month and three months post-delivery. Detailed results are shown in the supplementary Figure S1.

Hematological Alterations in Infants

At birth, infants of group 1 with prenatal AZT exposure showed significantly lower median hemoglobin (13.3 g/dl vs. 15.2 g/dl, $p<0.001$) and median RBC (3.7/µl vs. 4.5/µl, $p<0.001$) levels compared to those of group 2. The median MCV (106fl vs. 100fl, $p<0.001$) and RDW (17.1% vs. 16.2%, $p<0.01$) were significantly higher in infants exposed to AZT during the prenatal period. At birth, the overall frequency of anemia ≥ grade 1 was 26%, with a significantly higher frequency in infants with antenatal AZT exposure (47% group 1 vs. 11% group 2, $p<0.001$). Two infants with prenatal AZT exposure had severe (grade 3) or potentially life threatening (grade 4) anemia (6% of group 1-infants) at birth compared to zero in group 2.

The median hemoglobin concentrations declined in all infants from 14.3 (IQR: 13.0 to 15.5) g/dl at birth to 11.6 (IQR: 9.9 to 12.7) g/dl at one month of age and to 10.5 (IQR: 9.9 to 11.4) g/dl at three months of age. At one month (IQR: 31–36days) and at three months (IQR: 91–93 days) of age, differences in median hemoglobin and median RBC between both groups were no longer significant.

At one month of age, anemia ≥ grade 1 was observed in 29% of all infants (36% group 1 vs. 20% group 2, $p = 0.35$), with one of the group 1-infants (3%) showing severe anemia. At three month

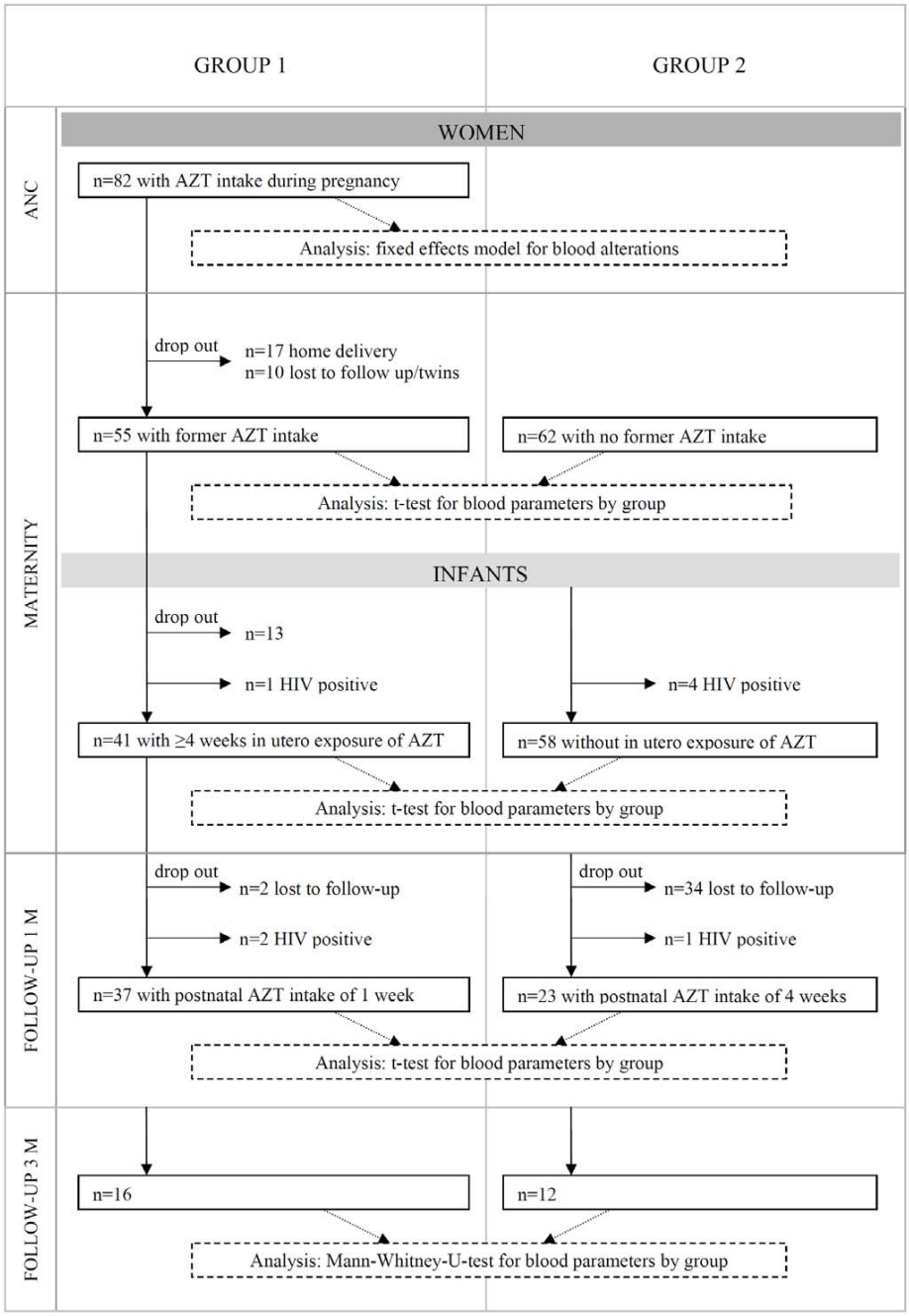

Figure 1. Flow chart of study cohort. Study population and applied statistical tests during antenatal care visits, delivery and follow-up visits at one month and three months post-delivery.
doi:10.1371/journal.pone.0055633.g001

of age, the overall anemia rate (≥ grade 1) was 58% (46% in group 1 vs. 73% in group 2-infants, $p = 0.24$).

The median granulocyte count at birth was significantly lower in infants with AZT exposure during pregnancy (5.0/nl in group 1 vs. 7.3/nl in group 2, $p<0.05$). At birth, granulocytopenia ≥ grade 1 was observed in 37% of all infants with a significantly higher frequency in group 1-infants (52% in group 1 vs. 26% in group 2, $p<0.05$). Toxicity ≥ grade 3 was observed in 18% of group 1-infants compared to 9% of group 2-infants, $p = 0.31$.

The overall median granulocyte count was 6.2 (IQR: 3.9–8.6)/nl and decreased to 2.8 (IQR: 1.6–3.4)/nl at one month of age and to 2.7 (IQR: 1.8–3.3)/nl at three months of age. At one month, the

Table 1. Baseline characteristics of mothers of group 1 and 2.

	Group 1	Group 2	p
	Enrolment in antenatal clinic/ pre-delivery AZT intake	Enrolment in maternity ward/no pre-delivery AZT intake	
Total number of pregnant women included	82	62	
Pregnancy week at start of AZT intake [median (IQR)]	29.1 (28.0–32.2)		
CD4 at enrolment in antenatal clinic [median (IQR) cells/μl)]	390 (267–515)		
Place of delivery [no. (%)]:			
Maternity ward	55 (67%)	62 (100%)	
Home delivery	17 (21%)[a]	0	
Lost to follow-up	10 (12%)[a]	0	
Marital status: married [%]:	74.5%	76.7%	0.96[b]
Years of education [median (IQR) years]	7 (7–7)	7 (7–7)	0.20[b]
Travel minutes to hospital [median (IQR)]	30 (30–60)	30 (30–60)	0.35
Household number [median (IQR)]	4 (3–5)	3 (2–5)	0.51
Number of children [median (IQR)]	2 (1–3)	1 (0.5–2)	0.40
Age at enrolment [median (IQR) years]	28 (24–30)	25 (23–29)	0.58
Weight at enrolment [median (IQR) kg]	60 (54–65)	57 (53–65)	0.64
Height [median (IQR) cm]	158 (154–160)	157 (152–160)	0.93
Gravida [median (IQR)]	3 (2–3)	3 (2–3)	0.61
Para [median (IQR)]	2 (1–2)	2 (1–3)	0.98

[a]Excluded from below socio-demographic comparison.
[b]Mann-Whitney-U-test; all other compared by t-test.
doi:10.1371/journal.pone.0055633.t001

Table 2. Baseline characteristics of infants of group 1 and 2.

	Group 1	Group 2	p
	Enrolment in antenatal clinic/ pre-delivery AZT intake	Enrolment in maternity ward/no pre-delivery AZT intake	
No AZT intake during pregnancy [no]		62	
At least four weeks antenatal AZT/no twins/available blood count results [no]	42		
HIV positive children at delivery [% (no)]	2.4% (1/41)	8.5% (4/47)	
Children enrolled in study [no]	41	58	
CD4 of mothers at delivery [median (IQR) cells/μl; no.]	350 (252–490); 25	262 (194–474); 40	0.26
Duration of prenatal AZT exposure [median (IQR) days]	57 (43–71)	0	
Duration of AZT syrup intake of infants [days]	7	28	
Mode of delivery: section [%]	7.5	11.1	0.41[b]
Preterm infants [%]	9.8	3.5	0.20[b]
Sex: female [%]	46.3	54.4	0.28[b]
Weight [median (IQR) g]	3100 (2840–3450)	3200 (2900–3500)	0.67
Apgar newborns at 1 minute	9 (8–9)	9 (8–9)	0.63[a]
Apgar newborns at 5 minutes	10 (10–10)	10 (10–10)	0.28[a]
Frequency of symptom maternal fever at delivery [%]	2.4	0	0.42
Median no. of sulphadoxine-pyrimeth. doses during pregnancy	2 (2–2)	2(1–2)	0.10[a]

[a]Mann-Whitney-U-test.
[b]Fisher's exact test; all other compared by t-test.
doi:10.1371/journal.pone.0055633.t002

median granulocyte count was significantly lower in group 2-infants compared to group 1-infants (3.2/nl in group 1 vs. 1.7/nl in group 2, $p = 0.001$). At this age, 19% of all infants presented with granulocytopenia ≥ grade 1, with a significantly higher frequency in group 2-infants (9% in group 1 vs. 33% in group 2, $p = 0.039$). Toxicity ≥ grade 3 was observed in 6% of group 1-infants and 10% of group 2-infants, $p = 1.0$.

The hematologic parameters of infants in group 1 and group 2 are summarized in table 3 and figure 2. Frequency of anemia and granulocytopenia are shown in table 4.

Transmission Rate

HIV-DNA PCR was performed in 88 infant blood samples at birth and in 63 infant blood samples at six weeks of age.

The *in utero* transmission rate tested at birth was 2.4% (CI: 0.35–16.0) in group 1-infants and 8.5% (CI: 3.3–21.1) in group 2-infants. The transmission rate estimated by Kaplan-Meier at six weeks of age was 7.7% in group 1 (CI: 2.5–22.1) and 12.5% (CI: 5.2–28.5) in group 2-infants.

Discussion

Focusing on hematological parameters in pregnant women and their infants up to the age of three months in a rural Tanzanian setting, this study was conducted to assess the impact of antiretroviral prophylaxis involving treatment of both mothers and infants with AZT.

Our evaluation was performed within the frame of an observational study in which all patients received antiretroviral prophylaxis as recommended by WHO guidelines. The two subgroups of our cohort were comparable with regard to the clinical and socio-demographic variables tested.

During AZT intake, pregnant women showed a significant decline in granulocyte count and RBC. Hemoglobin decreased within the first four weeks of AZT intake and then increased. Concurrently, a significant increase of MCV, RDW and platelet count was observed. However, at birth, there was no significant difference in hemoglobin between women taking AZT during pregnancy (group 1) and those did not (group 2). The values for MCV, RDW and platelet count were significantly higher in group 1-women at birth and RBC counts were significantly lower.

The transient decrease of hemoglobin is in agreement with previous publications. Briand *et al.* [8] demonstrated a suppression of hemoglobin levels in pregnant women taking AZT. Comparing short versus long exposure during pregnancy, a slight difference in hemoglobin level persisted until the time of delivery whereas differences in the other values resolved by this time.

It is known that platelet counts may generally decrease during pregnancy [21]. However, our fixed effects model showed an increase during AZT treatment of pregnant women, and a significantly higher platelet count in group 1- compared to group 2-women was observed at the time of delivery. This indicates that AZT treatment may increase platelet counts in HIV-infected pregnant women.

AZT has been shown to cross the placenta, reaching a cord-to-maternal blood level ratio of 0.8 [22] and to affect myeloid and erythroid cell lines [23], resulting in a decrease in hemoglobin levels and the number of granulocytes.

Our study found significantly lower hemoglobin levels in infants with intrauterine AZT exposure (group 1) at birth. The effect on hemoglobin was associated with a significantly higher frequency of

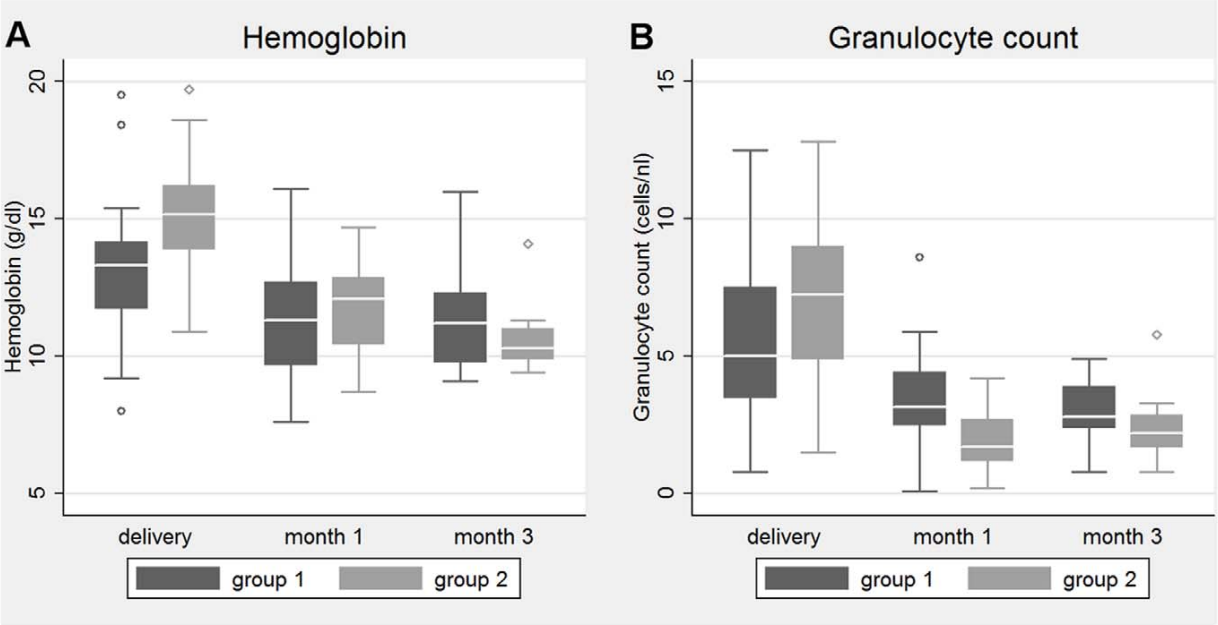

Figure 2. Hemoglobin and granulocyte count in infants at birth, one month and three months of age. A. At birth, group 1-infants with prenatal AZT exposure presented with significantly lower median hemoglobin (13.2 g/dl vs. 15.2 g/dl, $p<0.001$) than group 2-infants. At one month and at three months of age, differences in median hemoglobin between both groups were no longer significant. B. The median granulocyte count at birth was significantly lower in infants with AZT exposure during pregnancy (5.1/nl in group 1 vs. 7.3/nl in group 2, $p<0.05$). At one month of age, the median granulocyte count was significantly lower in group 2-infants compared to group 1-infants (3.2/nl in group 1 vs. 1.7/nl in group 2, $p = 0.001$). No statistically significant differences in granulocyte count were observed at three months of age.
doi:10.1371/journal.pone.0055633.g002

Table 3. Hematological parameters of infants by group at birth, 4–6 weeks of age and 12 weeks of age.

	Birth			Week 4–6 (IQR: 31–36; n = 62)			Week 12 (IQR: 91–93; n = 31)		
	group 1	group 2	p	group 1	group 2	p	group 1	group 2	P[a]
RBC, median	3.7 (3.2–4.0)	4.5 (4.2–4.8)	**<0.001**	3.6 (3.0–4.0)	3.7 (3.4–4.1)	0.58	4.4 (4.1–4.5)	4.1 (3.9–4.5)	0.18
(IQR), 10^6/uL			n = 78			n = 51			n = 24
Hemoglobin, median	13.3 (11.8–14.2)	15.2 (13.9–16.2)	**<0.001**	11.3 (9.7–12.7)	12.1 (10.5–12.9)	0.49	11.2 (9.8–12.3)	10.3 (9.9–11.0)	0.6
(IQR), g/dl			n = 78			n = 51			n = 24
MCV, median	106 (101–114)	100 (97–105)	**0.001**	95 (89–101)	92 (89–94)	0.27	74 (70–81)	75 (72–78)	0.47
(IQR), fl			n = 78			n = 51			n = 24
RDW, median	17.1 (16.2–18.1)	16.2 (15.6–16.9)	**0.008**	17.1 (16.8–18.3)	15.7 (14.8–17.1)	**0.002**	15.9 (15.6–16.9)	16.0 (14.6–16.6)	0.54
(IQR), %			n = 75			n = 51			n = 24
WBC, median	10.4 (8.2–13.6)	12.8 (10.9–17.1)	0.07	9.5 (7.6–11.5)	8.5 (6.3–10.9)	0.35	10.6 (8.8–14.7)	8.9 (6.4–11.4)	0.11
(IQR),/nL			n = 79			n = 53			n = 25
Granulocytes, median	5.0 (3.5–7.5)	7.3 (4.9–9.0)	**0.042**	3.2 (2.5–4.4)	1.7 (1.2–2.7)	**0.001**	2.8 (2.4–3.9)	2.2 (1.7–2.9)	0.18
(IQR),/nL			n = 79			n = 53			n = 25
Lymphocytes, median	4.0 (3.5–6.2)	4.9 (3.2–6.4)	0.42	4.8 (3.9–7.3)	5.3 (4.1–8.3)	0.62	6.8 (5.9–10.2)	6.1 (3.7–7.7)	0.18
(IQR),/nL			n = 79			n = 53			n = 25
Monocytes, median	1.0 (0.6–1.6)	1.3 (0.7–1.9)	0.42	0.9 (0.7–1.3)	0.9 (0.7–1.3)	0.73	0.8 (0.6–1.4)	0.8 (0.5–1.1)	0.16
(IQR),/nL			n = 79			n = 53			n = 25
Platelets, median	375 (303–436)	326 (245–386)	0.09	397 (248–493)	420 (216–533)	0.79	440 (284–568)	403 (215–569)	0.64
(IQR),/nL			n = 76			n = 49			n = 23

[a]Mann-Whitney-U-test, all other compared by t-test.

doi:10.1371/journal.pone.0055633.t003

anemia grade ≥1 at birth. These results agree with those of Connor *et al.* and Sperling *et al.* [12,14], who both showed significantly lower levels of hemoglobin in infants exposed *in utero* to AZT. In our study, differences in hemoglobin were no longer significant by the age of one month, which is most likely the result of differences in postnatal AZT intake and a faster decrease of hemoglobin in group 2-infants, again agreeing with Connor *et al.* and Sperling *et al.* The overall rate of anemia ≥ grade 1 was 29% with no significant difference between the groups at one month of age. In contrast, Briand *et al.* found significant differences in the frequency of anemia between infants with three days or six weeks of postnatal AZT intake at the age of six weeks. Interestingly, this effect was found to be independent of *in utero* exposure [24].

At birth, infants of group 1 had significantly lower granulocyte counts, accompanied by a significantly higher frequency of granulocytopenia ≥ grade 1. This effect of *in utero* exposure to AZT in infants, including persistence of decreased granulocyte counts up to 18 months after birth, has been described previously [11].

Table 4. Frequency of anemia and granulocytopenia in infants of group 1 and 2.

	Birth (n = 78)			Month 1 (n = 51)			Month 3 (n = 24)		
Anemia	**group 1**	**group 2**	**P[a]**	**group 1**	**group 2**	**P[a]**	**group 1**	**group 2**	**P[a]**
grade ≥1	15 (46.9%)	5 (10.9%)	**0.001**	11 (35.5%)	4 (20.0%)	0.35	6 (46.2%)	8 (72.7%)	0.24
grade ≥3	2 (06.3%)	0 (00.0%)	0.17	1 (03.2%)	0 (00.0%)	1	0 (00.0%)	0 (00.0%)	1
	birth (n = 79)			**month 1 (n = 53)**			**month 3 (n = 25)**		
Granulocytopenia	**group 1**	**group 2**	**P[a]**	**group 1**	**group 2**	**P[a]**	**group 1**	**group 2**	**P[a]**
grade ≥1	17 (51.5%)	12 (26.1%)	**0.033**	3 (09.4%)	7 (33.3%)	**0.039**	1 (07.7%)	2 (16.7%)	0.59
grade ≥3	6 (18.2%)	4 (08.7%)	0.31	2 (06.3%)	2 (09.5%)	1	0 (00.0%)	0 (00.0%)	1

[a]Fisher's exact test.

doi:10.1371/journal.pone.0055633.t004

After receiving AZT for one month in the postnatal period, group 2-infants had a significantly lower granulocyte count and significantly higher frequency of granulocytopenia ≥1 than group 1-infants having a postnatal AZT intake of just one week. By the age of one month, 9% of infants had developed a severe granulocytopenia (≥ grade 3), most likely due to *in utero* and postpartal exposure to AZT.

Throughout this study, alterations in the mothers' and infants' blood parameters were carefully monitored. However, it must be kept in mind that the routine health care in low-income countries only rarely includes monitoring of hemoglobin and granulocyte counts during AZT-intake. Despite PCR-HIV testing of infants blood samples at the age of one month being common, economic or logistic constraints preclude the regular monitoring of blood components. It is therefore important to include this lack of monitoring in debates about the potential toxicity of ARVs in the context of peripheral, resource-limited settings.

The transmission rate estimated by Kaplan-Meier was 7.7% in group 1 and 12.5% in group 2-infants at 6 weeks of age. Two randomized, double-blind, placebo-controlled trials have shown that AZT administered in the last four weeks of pregnancy is effective in reducing the transmission rate to 14.7% compared to 24.8% with placebo at six weeks of age [25]. Dabis *et al.* [6] have shown that combining the four-week course of AZT with sdNVP further reduces the six week probability of transmission to 6.5%, a finding largely consistent with our own.

To ensure comparability of the two groups, we checked for differences in disease progression in terms of CD4-cell counts, as well as with regards to socio-demographic aspects such as age, weight, marital status or years of education and found that none of these factors differed significantly between groups 1 and 2. Nevertheless, it cannot be ruled out that there may be other unconsidered factors such as health-seeking behavior that might vary between the two groups and act as confounders.

At the point of birth, group 2-infants, who had no prior AZT exposure during pregnancy, represent a true control group for analyzing the influence on group 1- infants of AZT exposure during pregnancy. However, during follow-up visits at one and three months of age, infants of both groups had different pre- as well as postnatal ARV exposure and are not strictly comparable. It is a limitation of this study that alterations in blood parameters at these time points can therefore not explicitly be assigned to either part of the regimen. However, establishing a control group with no AZT exposure at all would have exceeded the frame of a study that was primarily aimed at describing the hematological effects of PMTCT regimens actually administered in practice.

A further limitation of our study is the high drop-out rate of infants throughout the follow-up period. Loss to follow-up is a common problem for PMTCT services in resource-limited settings [26,27]. In addition to the parents failing to understand the importance of follow-up visits, especially in the absence of illness, infant death is a major reason for loss to follow-up, as described by Ahoua *et al.* [28]. In our study, no information about the development of infants lost to follow-up exists and cases of undiagnosed virus transmission, side effects or mortality among such infants cannot be ruled out.

In conclusion, this study revealed that AZT exposure during and after pregnancy can cause significant hematologic alterations in women and infants. However, it was also shown that the side effects observed were generally transient and predominantly mild in nature. However, the few cases of severe hematologic toxicity (≥ grade 3) in infants should be taken into serious consideration when planning and implementing antiretroviral PMTCT interventions in structure-limited settings, where surveillance of blood alterations is often not standard practice. Our results demonstrate that monitoring side effects during antiretroviral PMTCT regimens is a crucial factor for intervention safety, and should be a constituent part for any such services. Further research involving larger cohorts and longer follow-up periods are needed to further analyze the impact of regimens involving AZT on maternal and infant health.

Supporting Information

Figure S1 Blood values in women of groups 1 and 2 during AZT intake Figures show selected blood values in group 1- and 2-women during AZT intake. Time points are: initiation of AZT intake (day 0), 7^{th}, 14^{th}, 21^{st}, 28^{th} day of AZT intake, delivery and one month post-delivery. The period between 28^{th} day of AZT intake and delivery differs between women, depending on the gestational stage at initiation of AZT. Group 1-women had antenatal AZT intake, group 2-women were included at delivery and had no antenatal AZT intake. Sample sizes were: n≥70 at beginning, n≥70 at 7^{th}day, n≥56 at 14^{th}day, n≥62 at 21^{st}day, n≥41 at 28^{th} day of AZT intake. At delivery, sample sizes were n≥30 (group 1-women) and n≥54 (group 2 women); at one month post-delivery, sample sizes were n≥39 (group 1-women) and n≥26 (group 2-women). A. A decrease and a subsequent increase in hemoglobin values was shown in group 1- women. No significant difference between the median hemoglobin levels of group 1- and 2-women was observed at birth and one month post-delivery. B. Mean corpuscular volume increased with AZT intake and resulted in statistically significant higher values at delivery (87fl vs. 79fl, $p<0.001$) in group 1-women. C. Red blood count decreased in the first weeks of AZT intake. Median red blood count was significantly lower in group 1-women (3.66/µl vs. 4.04/µl, $p<0.05$) at delivery. There was no statistically significant difference at 1 month post-delivery. D. Platelet count increased during the time of AZT intake. At delivery, the median platelet count was significantly higher in women with antenatal AZT intake (367.000/mm^3 vs. 273.500/mm^3, $p<0.05$), although by one month post-delivery the difference was no longer significant. E and F. White blood counts and granulocyte counts decreased during AZT intake. Comparing group 1 and 2 at delivery and one month post-delivery, differences in the median white blood count and median granulocyte count were not statistically significant.
(TIF)

Acknowledgments

The authors would like to express their gratitude to all staff of Kyela District Hospital and their patients, the District Medical Officer, the Regional Health Management Team and the National AIDS Control Program in Tanzania.

Author Contributions

Revision of manuscript: JZ AK JS IL FD AH WK GH ST. Conceived and designed the experiments: JZ AK JS IL FD GH ST. Performed the experiments: JZ JS IL FD. Analyzed the data: JZ AK IL AH WK ST. Contributed reagents/materials/analysis tools: JZ AK JS FD GH. Wrote the paper: JZ AK JS IL FD AH WK GH ST.

References

1. Dao H, Mofenson LM, Ekpini R, Gilks CF, Barnhart M, et al. (2007) International recommendations on antiretroviral drugs for treatment of HIV-infected women and prevention of mother-to-child HIV transmission in resource-limited settings: 2006 update. Am J Obstet Gynecol 197: S42–55.

2. Mofenson LM (2010) Prevention in neglected subpopulations: prevention of mother-to-child transmission of HIV infection. Clin Infect Dis 50 Suppl 3: S130–148.
3. World Health Organization (2011) Global HIV/AIDS Response. Epidemic update and health sector progress towards Universal Access. Progress Report 2011. Available: http://www.who.int/hiv/pub/progress_report2011/summary_en.pdf. Accessed 2013 Jan 8.
4. Eshleman SH, Guay LA, Mwatha A, Brown E, Musoke P, et al. (2005) Comparison of mother-to-child transmission rates in Ugandan women with subtype A versus D HIV-1 who received single-dose nevirapine prophylaxis: HIV Network For Prevention Trials 012. J Acquir Immune Defic Syndr 39: 593–597.
5. McIntyre JA, Hopley M, Moodley D, Eklund M, Gray GE, et al. (2009) Efficacy of short-course AZT plus 3TC to reduce nevirapine resistance in the prevention of mother-to-child HIV transmission: a randomized clinical trial. PLoS Med 6: e1000172.
6. Dabis F, Bequet L, Ekouevi DK, Viho I, Rouet F, et al. (2005) Field efficacy of zidovudine, lamivudine and single-dose nevirapine to prevent peripartum HIV transmission. AIDS 19: 309–318.
7. Kirsten I, Sewangi J, Kunz A, Dugange F, Ziske J, et al. (2010) Adherence to combination prophylaxis for prevention of mother-to-child-transmission of HIV in Tanzania. PLoS One 6: e21020.
8. Briand N, Lallemant M, Jourdain G, Techapalokul S, Tunthanathip P, et al. (2007) Haematological safety of perinatal zidovudine in pregnant HIV-1-infected women in Thailand: secondary analysis of a randomized trial. PLoS Clin Trials 2: e11.
9. Sinha G, Choi TJ, Nayak U, Gupta A, Nair S, et al. (2007) Clinically significant anemia in HIV-infected pregnant women in India is not a major barrier to zidovudine use for prevention of maternal-to-child transmission. J Acquir Immune Defic Syndr 45: 210–217.
10. Feiterna-Sperling C, Weizsaecker K, Buhrer C, Casteleyn S, Loui A, et al. (2007) Hematologic effects of maternal antiretroviral therapy and transmission prophylaxis in HIV-1-exposed uninfected newborn infants. J Acquir Immune Defic Syndr 45: 43–51.
11. Le Chenadec J, Mayaux MJ, Guihenneuc-Jouyaux C, Blanche S (2003) Perinatal antiretroviral treatment and hematopoiesis in HIV-uninfected infants. AIDS 17: 2053–2061.
12. Connor EM, Sperling RS, Gelber R, Kiselev P, Scott G, et al. (1994) Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. N Engl J Med 331: 1173–1180.
13. Pacheco SE, McIntosh K, Lu M, Mofenson LM, Diaz C, et al. (2006) Effect of perinatal antiretroviral drug exposure on hematologic values in HIV-uninfected children: An analysis of the women and infants transmission study. J Infect Dis 194: 1089–1097.
14. Sperling RS, Shapiro DE, McSherry GD, Britto P, Cunningham BE, et al. (1998) Safety of the maternal-infant zidovudine regimen utilized in the Pediatric AIDS Clinical Trial Group 076 Study. AIDS 12: 1805–1813.
15. United Nations Development Programme (2011) Human Development Report 2011. Sustainability and Equity: A Better Future for All. Available: http://hdr.undp.org/en/media/HDR_2011_EN_Complete.pdf. Accessed 2013 Jan 8.
16. UNAIDS/WHO Working Group on Global HIV/AIDS and STI Surveillance (2008) United Republic of Tanzania: Epidemiological Fact Sheet on HIV and AIDS. Available: http://apps.who.int/globalatlas/predefinedReports/EFS2008/full/EFS2008_TZ.pdf. Accessed 2013 Jan 8.
17. Tanzania Commission for AIDS (TACAIDS), National Bureau of Statistics (NBS) (2008) Tanzania HIV/AIDS and Malaria Indicator Survey 2007-08. Available: http://www.tacaids.go.tz/dmdocuments/THMIS%202007-08.pdf. Accessed 2013 Jan 8.
18. National Institute for Allergy and Infectious Disease (NIAID), Division of AIDS (2009) Table for grading the severity of adult and pediatric adverse events, version 1.0 (2004), Clarification August 2009. Available: http://rsc.tech-res.com/Document/safetyandpharmacovigilance/Table_for_Grading_Severity_of_Adult_Pediatric_Adverse_Events.pdf. Accessed 2013 Jan 8.
19. Alioum A, Dabis F, Dequae-Merchadou L, Haverkamp G, Hudgens M, et al. (2001) Estimating the efficacy of interventions to prevent mother-to-child transmission of HIV in breast-feeding populations: development of a consensus methodology. Stat Med 20: 3539–3556.
20. Alioum A, Cortina-Borja M, Dabis F, Dequae-Merchadou L, Haverkamp G, et al. (2003) Estimating the efficacy of interventions to prevent mother-to-child transmission of human immunodeficiency virus in breastfeeding populations: comparing statistical methods. Am J Epidemiol 158: 596–605.
21. Sill PR, Lind T, Walker W (1985) Platelet values during normal pregnancy. Br J Obstet Gynaecol 92: 480–483.
22. Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission (2011) Recommendations for Use of Antiretroviral Drugs in Pregnant HIV-1-Infected Women for Maternal health and Interventions to Reduce Perinatal HIV Transmission in the United States. 1–207. Available: http://aidsinfo.nih.gov/contentfiles/lvguidelines/perinatalgl.pdf. Accessed 2013 Jan 8.
23. Shah MM, Li Y, Christensen RD (1996) Effects of perinatal zidovudine on hematopoiesis: a comparison of effects on progenitors from human fetuses versus mothers. AIDS 10: 1239–1247.
24. Briand N, Le Coeur S, Jourdain G, Hotrawarikarn S, Sirinontakan S, et al. (2010) Hematological safety of perinatal exposure to zidovudine in uninfected infants born to HIV type 1-infected women in Thailand. AIDS Res Hum Retroviruses 26: 1163–1166.
25. Leroy V, Karon JM, Alioum A, Ekpini ER, Meda N, et al. (2002) Twenty-four month efficacy of a maternal short-course zidovudine regimen to prevent mother-to-child transmission of HIV-1 in West Africa. AIDS 16: 631–641.
26. Manzi M, Zachariah R, Teck R, Buhendwa L, Kazima J, et al. (2005) High acceptability of voluntary counselling and HIV-testing but unacceptable loss to follow up in a prevention of mother-to-child HIV transmission programme in rural Malawi: scaling-up requires a different way of acting. Trop Med Int Health 10: 1242–1250.
27. Mirkuzie AH, Hinderaker SG, Sisay MM, Moland KM, Morkve O (2011) Current status of medication adherence and infant follow up in the prevention of mother to child HIV transmission programme in Addis Ababa: a cohort study. J Int AIDS Soc 14: 50.
28. Ahoua L, Ayikoru H, Gnauck K, Odaru G, Odar E, et al. (2010) Evaluation of a 5-year programme to prevent mother-to-child transmission of HIV infection in Northern Uganda. J Trop Pediatr 56: 43–52.

Lebenslauf

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Publikationsliste

Originalarbeiten, peer-reviewed Journals:

- **Kirsten I**, Sewangi J, Kunz A, Dugange F, Ziske J, Jordan-Hader B, Harms G, Theuring S. *Adherence to Combination Prophylaxis for Prevention of Mother-to-Child-Transmission of HIV in Tanzania*. PLoS ONE 2011;6(6):e21020. doi:10.1371/journal.pone.0021020.
- Hauser A, Sewangi J, Mbezi P, Dugange F, **Lau I**, Ziske J, Theuring S, Kuecherer C, Harms G, Kunz A. *Emergence of Minor Drug-Resistant HIV-1 Variants after Triple Antiretroviral Prophylaxis for Prevention of Vertical HIV-1 Transmission.* PLoS ONE 2012;7(2):e32055. doi:10.1371/journal.pone.0032055.
- Kunz A, von Wurmb-Schwark N, Sewangi J, Ziske J, **Lau I**, Mbezi P, Theuring S, Hauser A, Dugange F, Katerna A, Harms G. Zidovudine *Exposure in HIV-1 Infected Tanzanian Women Increases Mitochondrial DNA Levels in Placenta and Umbilical Cords*. PLoS ONE 2012;7(7):e41637. doi:10.1371/journal.pone.0041637.
- Ziske J, Kunz A, Sewangi J, **Lau I,** Dugange F, Hauser A, Kirschner W, Harms G, Theuring S. *Hematological changes in women and infants exposed to AZT-containing regimen for prevention of mother-to-child-transmission of HIV in Tanzania.* PLoS ONE 2013;8(2):e55633. doi:10.1371/journal.pone.0055633.

Weitere Publikationen:

- Müller A und **Kirsten I**. *Menschenrecht gegen Menschenrecht*. Forum Recht, 2010;4:117-120.

Konferenzbeiträge:

- **Kirsten I**. *Implementation of new Tanzanian PMTCT-guidelines: a field study and report.* 1st World Health Summit, Charité Berlin 2009; Student panel. (Vortrag)
- Theuring S, Sewangi J, **Kirsten I**, Dugange F, Ziske J, Kunz A, Pilger D, Jordan-Harder B, Harms G. *PMTCT combination prophylaxis: The challenge of adherence to a complex regimen during pregnancy in Kyela, Tanzania.* 18th International AIDS Conference, Vienna 2010; MOPE0267. (Abstract und Poster)
- Theuring S, **Kirsten I**, Sewangi J, Dugange F, Ziske J, Kunz A, Jordan-Harder B, Hauser A, Harms G. *Adherence to combination prophylaxis throughout all stages of PMTCT intervention is difficult to achieve in a peripheral Tanzanian setting.* 6th IAS Conference on HIV Pathogenesis, Treatment and Prevention, Rome 2011; TUPE276. (Abstract und Poster)
- Theuring S, **Kirsten I**, Sewangi J, Dugange F, Ziske J, Kunz A, Jordan-Harder B, Hauser A, Harms G. *Prevention of mother-to-child transmission of HIV: Adherence to combination prophylaxis is difficult to achieve in a peripheral Tanzanian setting.* Trop Med Int Health (Abstracts of the 7th Congress on Tropical Medicine and Interantional Health) 2011;16 Suppl 1:82. (Abstract)
- Hauser A, Sewangi J, Dugange F, **Kirsten I**, Ziske J, Theuring S, Kuecherer C, Harms G, Kunz A. *Emergence of minor drug-resistant HIV-1 variants in Tanzanian women after triple antiretroviral prophylaxis using zidovudine, lamivudine and nevirapine for the prevention of mother-to-child-transmission of HIV-1*. Trop Med Int Health (Abstracts of the 7th Congress on Tropical Medicine and Interantional Health) 2011;16 Suppl 1:82. (Abstract)

- Ziske J, Sewangi J, Dugange F, **Kirsten I,** Harms G, Theuring S, Kunz A. *Hematological changes in infants exposed to AZT-containing prophylaxis for prevention of mother-to-child-transmission of HIV-1 in Tanzania.* Trop Med Int Health (Abstracts of the 7th Congress on Tropical Medicine and International Health) 2011;16 Suppl 1:85. (Abstract)
- Hauser A, Kuecherer C, Theuring S, Sewangi J, Dugange F, Mbezi P, **Lau I**, Ziske J, Harms G, Kunz A. *Detection of drug-resistant HIV-1 variants in cellular DNA of Tanzanian women following antiretroviral prophylaxis for vertical transmission.* 10th European meeting on HIV & Hepatitis – Treatment Strategies & Antiviral Drug Resistance, Barcelona 2012; P_39. (Abstract und Poster)

Danksagung

Diese Arbeit ist Mrs. Florence Mtanga gewidmet, ohne deren Engagement die umfangreiche Datenerhebung für diese Studie nicht möglich gewesen wäre. Sie verstarb im März 2012 in Tansania, doch in meinen Erinnerungen ist sie als fröhliche und energische „Mama Mtanga“ noch immer lebendig. Mungu awabariki!

Allen voran danke ich Frau Prof. Gundel Harms zutiefst für die engagierte Betreuung der Arbeit und die Geduld, die sie dabei bewies. Auf ihre fachliche Kompetenz und ihre zeitnahe Beantwortung aller Fragen konnte ich mich stets verlassen. Mein herzlicher Dank gilt des Weiteren den Projektkoordinatorinnen Dr. Andrea Kunz und Dr. Stefanie Theuring, die mich bestmöglich unterstützten und stets als Ansprechpartnerinnen zur Verfügung standen – Dr. Kunz während der Vorbereitungen und der Datenerhebung in Tansania und Dr. Theuring während der Datenanalyse und der Manuskripterstellung in Deutschland. Während des langwierigen Auswertungsprozesses verstand Dr. Theuring es, mich mit ihrer positiven Art immer wieder aufs Neue zu motivieren. Meiner Mitdoktorandin Judith Ziske danke ich für die kollegiale Zusammenarbeit und wünsche ihr von ganzem Herzen gutes Gelingen beim Erstellen ihrer eigenen Dissertation. Dr. Brigitte Jordan-Harder gilt mein Dank für den wunderbaren Empfang und ihre Unterstützung während der Forschungsaufenthalte in Tansania. Dr. Andrea Hauser hatte stets ein offenes Ohr für meine Gedanken und Sorgen, und ich danke ihr sehr für alle fachlichen Erklärungen und empathischen Gespräche.

Allen Kollegen und Kolleginnen des Kyela Distrikt Hospital, die sich mit viel Fleiß und Engagement der Datenerhebung widmeten, insbesondere „Mama“ Ipambalaga und Renatus Msigwa gebührt mein herzlicher Dank für die großartige Zusammenarbeit. Auch Dr. Julius Sewangi und Dr. Festo Dugange möchte ich für Ihre fortwährende Unterstützung danken, mit der sie maßgeblich zum Gelingen des Projektes beigetragen haben. Saskia Morgenstern danke ich für ihre ehrenamtliche Hilfe bei der Datenerhebung und Sekela für ihre Mithilfe im Labor. Auch bei allen weiteren nicht namentlich erwähnten Co-Autoren und Helfern bedanke ich mich sehr für ihren beachtlichen Einsatz. Nicht zuletzt fühle ich mich allen Studienteilnehmerinnen und ihren Kindern zu tiefem Dank verpflichtet!

Dem Deutschen Akademischen Austauschdienst (DAAD) danke ich für die finanzielle Unterstützung während meines Forschungsaufenthaltes in Tansania.

Es gab viele weitere Menschen, die mir während der Forschungszeit in Kyela auf unterschiedlichste Weise beigestanden haben, sei es als Kollegen, als Freunde oder einfach als flüchtige Bekannte. Ihnen allen danke ich für ihre tatkräftige Hilfe und ihre Geduld, wenn es darum ging mir Kiswahili und die afrikanische Kultur näher zu bringen. Sie haben die Zeit in Kyela zu dem werden lassen, was sie für mich war: eine großartige Erfahrung mit dem Gefühl, in diesem kleinen unscheinbaren tansanischen Ort ein Stück zu Hause gefunden zu haben.

Nicht zuletzt bin ich meiner Familie für die großartige Unterstützung von ganzem Herzen zum Dank verpflichtet. Mein geliebter Mann stand mir während allen Phasen der Entstehung dieser Arbeit mit Rat, Tat und Zuspruch zur Seite und hat mir bei allen IT-Problemen geduldig geholfen. Meiner Tochter Naima danke ich für die Stunden, die sie mich – wenn auch nicht immer freiwillig – am Schreibtisch hat arbeiten lassen und dafür, dass sie mir täglich zeigt, wie viel Spannendes es noch zu entdecken gibt. Danke!