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PHARMACOVIGILANCE OF MASS DRUG ADMINISTRATION AS PREVENTIVE CHEMOTHERAPY TO CONTROL AND ELIMINATE LYMPHATIC FILARIASIS IN TANZANIA

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Pharmacovigilance of mass drug administration as preventive chemotherapy to control and eliminate lymphatic filariasis in Tanzania

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I dedicate this piece of work to my family, friends, relatives, office mates, and those with a positive and persevering attitude!

Popular science summary of the thesis

Lymphatic filariasis (LF) is a neglected tropical disease (NTD) particularly affecting populations in Sub-Saharan Africa (SSA). Efforts to combat this disease have been ongoing through mass drug administration (MDA) campaigns, deploying the use of ivermectin and albendazole (IA), but the safety and effectiveness of these drugs remain under scrutiny. My doctoral research project aimed to address these concerns by assessing the prevalence of LF and evaluating the safety and efficacy of the drugs used during MDA in Tanzania.

The safety and efficacy of marketed drugs need to be ascertained through a pharmacovigilance (PV) system throughout the product's life cycle. One major challenge facing SSA is the lack of a robust PV system to monitor the adverse effects of the drugs, particularly in populations with overlapping diseases like onchocerciasis or loiasis. To tackle this, I investigated the safety of MDA drugs (i.e., ivermectin and albendazole), by actively monitoring individuals in the Mkinga district, Tanga region. I assessed their response to IA and identified common adverse effects such as headache, drowsiness, fever, and dizziness. Importantly, I found that certain risk factors like pre-existing clinical symptoms increased the likelihood of experiencing adverse effects.

Furthermore, I examined changes in haematological and biochemical parameters following drug administration and determined that they do change after drug intake. The observed abnormalities need further evaluation for better management of AEs including ascertaining the safety of MDA drugs in future campaigns.

I further modelled the pharmacokinetics (PK) of ivermectin to understand drug exposure variability and highlighted the need for tailored dosing strategies, especially considering the observed variability in dosage when using height scales for measurement.

Despite the effectiveness of IA in clearing microfilariae (mf) from the blood, LF prevalence in Tanzania remains high, suggesting the need for alternative treatments targeting adult worms. Moving forward, integrating PV into the NTD programme and exploring alternative drug combinations could be a crucial step toward eliminating LF in affected regions.

Abstract

Mass drug administration (MDA) campaigns are usually conducted on an annual basis by many countries globally where neglected tropical diseases (NTDs), including lymphatic filariasis (LF), are endemic. Such campaigns are organized and executed by the Neglected Tropical Diseases Control Programmes (NTDCP) or the National Programmes for Elimination of LF (NPELF) whichever is applicable. During such campaigns, drugs are normally distributed randomly to at-risk populations to halt the transmission of diseases. It has been proven through research and evidence gathered through the World Health Organization (WHO) and NTDCP that such a move helps to stop the spreading of LF in affected communities. Nevertheless, in these campaigns, individuals subjected to such preventive chemotherapy are in all cases not tested for LF diagnosis before drug administration. Depending on disease prevalence, elimination status, distribution coverage, and availability of resources, such campaigns may be conducted once or twice a year.

During or after these campaigns evidence shows that, the concept of pharmacovigilance is not being considered in most of the cases and that drugs are distributed randomly without collecting safety information to be certain that the same are not causing any harm to exposed individuals. Pharmacovigilance (i.e., *the science and activities relating to the detection, assessment, understanding, and prevention of adverse effects or any other medicine or vaccine-related problem*) is still weak in many resource-constrained countries. Such countries are to-date grappling to set up systems that would allow for the effective collection of data on drug-related adverse events (AEs) and reactions (ADRs). Under-reporting is one of the mounting challenges and National Medicines Regulatory Authorities (NMRAs) do not have adequate resources or machinery to counter the status quo. Long-term and chronic undesirable events due to prolonged use of MDA drugs including those affecting the liver and kidneys are even of more concern.

In this PhD programme, we began by conducting a study to determine the prevalence and correlates of LF infection in the Mkinga district which is located in the Tanga region on the offshores of the Indian Ocean - the northeastern part of Tanzania (***Paper I***). The rationale of conducting such a study was to find out the level of antigenemia and microfilaremia as the same have an impact on the safety profile of drugs used for NTDs including LF (i.e., ivermectin and albendazole – IA). In this cross-sectional community-based survey, a total of 4115 individuals (49.7% males, 35.2% children) were screened for circulating filarial antigens (CFA), microfilaremia (mf), and disease manifestations in 15 villages between November 2018 and January 2019. MDA uptake in the previous year (2017) was also assessed. The overall prevalence of CFA-positivity was 5.8% (239/4115; 95% CI: 5.1–6.6), with significant heterogeneity seen between villages (range 1.2% to 13.5%). CFA-positivity was higher in males (8.8%) than females (3.3%) and correlated with increasing age ($p < 0.001$). Prevalence of mf amongst CFA-positives was 5.2%. The MDA uptake was only 60%. Prevalence of scrotal enlargement, hydrocele, swelling of arms or legs, lymphoedema, and lymphadenopathy was 6.4%, 3.7%, 1.35%, 1.2% and 0.32%, respectively. This study showed that the elimination target of <1% mf and <2% antigenemia to levels where recrudescence is unlikely to occur was not attained as compared to the baseline data. However, after 16 rounds of MDA, LF transmission was significantly reduced.

Following the prevalence study, we went on to conduct the safety surveillance study (***Paper II***) to document AEs that occurred following IA MDA. In this study, we identified the type, incidence, and associated risk factors. Around 9,640 eligible individuals received single-dose IA combination preventive chemotherapy and treatment-associated AEs were actively monitored through house-to-house visits on day 1, day 2, and day 7 of MDA. After MDA, 9288 participants (96.3%) were followed up of whom 442 reported 719 MDA-associated AEs. The incidence of experiencing one or more types of MDA-associated AE was 4.8% (95% CI = 4.3–5.2%); this was significantly higher among those with pre-MDA clinical events than those without (8.5% versus 4.1%, $p < 0.001$). AEs were mild (83.8%), moderate (15.9%), and severe (0.3%), and most resolved within 72 hours. The incidence of

experiencing one, two, and ≥ three types of AEs were 2.8%, 1.3%, and 0.6%, respectively. The most common AEs were headache (1.23%), drowsiness (1.15%), fever (1.12%), and dizziness (1.06%). Chronic illness, clinical manifestation of LF, being female, or having pre-existing clinical symptoms were significant predictors of AEs. Therefore, safety monitoring in individuals with underlying clinical conditions was recommended for timely detection and management of AEs during MDA campaigns.

To further ascertain the safety of MDA drugs in blood, kidneys, and liver, we extracted data to measure the haematological and biochemical parameters to determine their changing patterns (***Paper III***). In so doing we also examined their relationship with those who had experienced AEs in the previous safety study. In this nested analytical prospective study, we assessed data amongst 499 eligible individuals whose blood samples were collected before and after MDA. We measured Complete Blood Count (CBC) and renal and liver function tests at days 0 and 7 following MDA. After a comprehensive assessment of all blood indices as well as kidney and liver surrogate markers, we demonstrated that haematological and biochemical changes may occur following IA MDA. The median values of haematological parameters, including RBC, Hb, and HCT decreased while MCH, MCHC, and monocytes increased significantly in both CFA-positive and negative individuals ($p<0.05$). Median platelet counts, including MPV and PLCR, increased after MDA ($p<0.05$). Biochemical parameters, including ALT, AST, BilD, and BildT, showed varied results. Higher creatinine levels were observed, and a significant proportion of individuals had haematological and biochemical parameters below the reference range. Drowsiness was the most observed AE among individuals with abnormal parameters, followed by fever, dizziness, and nausea.

In **Paper IV**, a pharmacokinetic (PK) study was conducted to determine the PK properties of IVM in humans. The study was done to characterize the disposition of IVM and determine predictors of its PK for dose optimization during MDA. This was also a nested study in a bigger safety study in which data was evaluated amongst 468 individuals. PK samples were collected at 0, 2, 4, and 6 hours from

individuals weighing > 15 Kg receiving IVM (3-, 6-, 9-, or 12 mg) and ALB (400 mg) during an MDA campaign. Individual characteristics were assessed including demographics, laboratory/clinical parameters, and genetic variations of selected drug-metabolizing enzymes and transporters. IVM plasma concentrations were quantified by LC-MS/MS and analysed using population-pharmacokinetic (POPPK) modelling. A two-compartment model with transit absorption kinetics, and allometrically scaled oral clearance (CL/F) and central volume (Vc/F) were adopted. Fitting of the model to the current data identified a 48% higher bioavailability for the 3 mg dose compared to other doses and further identified a subpopulation with 97% higher mean transit time (MTT). The final estimates for CL/F, Vc/F, inter-compartment clearance (Q), peripheral volume (Vp), MTT, and absorption rate constant (Ka) for a 70 Kg person (on a dose other than 3 mg) were 7.7 L/h, 147 L, 20.4 L/h, 207 L, 1.5 h, and 0.71/h, respectively. Simulations indicated that weight-based dosing provides comparable exposure across weight bands, but height-based dosing with a capping IVM dose at 12 mg for individuals with height > 160cm under-doses those weighing > 70 Kg. The variability in IVM PK is partly explained by body weight and dose. The established POPPK model can be used for IVM dose optimization. Height-based pole dosing may result in varying IVM exposure of individuals in different weight bands; hence use of weighing scales for IVM dosing during MDA is recommended.

In **Paper V**, we investigated the efficacy of IA in clearing mf and reducing CFA levels in individuals. This community-based prospective study assessed the efficacy of MDA drugs in mf clearance and CFA reduction on days 7- and 6 months following MDA. The study was done in the same Mkinga district, Tanga region between November 2018 and June 2019. The status of mf and CFA on day 7 and six-month post-MDA was monitored. The primary efficacy outcomes were the clearance rates of mf on day 7 and six months, and CFA at 6 months of post-MDA. Out of 4,115 individuals screened, 239 (5.8%) tested positive for CFA, with 11 (4.6%) also positive for mf. The McNemar test revealed a significant improvement in mf clearance on day 7 following MDA (p=0.02). Out of 183 CFA-positive individuals who were available at 6-month follow-up, 160 (87.4%) remained CFA positive,

while 23 became CFA negative. The CFA clearance rate at 6 months post-MDA was 12.6% (95% CI = 8.52 – 18.5%). There was no significant association of variability in IVM plasma exposure (Cmax and AUC) with post-MDA mf or CFA clearance status. We concluded that preventive chemotherapy with IA effectively clears mf within a week, but the same drugs are ineffective in clearing CFA at six months post-MDA. We recommended alternative drug combinations targeting adult worms as IA is not macrofilaricidal.

In **conclusion**, LF has not been fully eliminated from the study area and the prevalence of the disease is still above the threshold recommended by WHO. MDA drugs are relatively safe for use during MDA. However, chronic illnesses, previous clinical manifestations of LF, being female, or having pre-existing clinical symptoms are significant predictors of AEs. Individuals exposed to MDA might experience changes in haematological and biochemical parameters after drug intake. The NTD programme should consider these findings when monitoring individuals taking part in future MDA campaigns. POPPK modelling can be used for IVM dose optimization and whenever possible, the programme should consider using weighing balances rather than height-poles for dose estimation when administering MDA drugs to individuals in endemic communities. Despite that the MDA drugs are still efficacious, alternative combinations targeting adult worms should be sought by the NTD programme.

List of scientific papers

I. **Fimbo AM,** Minzi OMS, Mmbando BP, Barry A, Nkayamba AF, Mwamwitwa KW, Malishee A, Seth MD, Makunde WH, Gurumurthy P, Lusingu JPA, Kamuhabwa AAR, Aklillu E. Prevalence and Correlates of Lymphatic Filariasis Infection and Its Morbidity Following Mass Ivermectin and Albendazole Administration in Mkinga District, North-Eastern Tanzania. J Clin Med. 2020 May 21;9(5):1550. doi: 10.3390/jcm9051550. PMID: 32455556; PMCID: PMC7290598.

II. **Fimbo AM,** Minzi OM, Mmbando BP, Gurumurthy P, Kamuhabwa AAR, Aklillu E. Safety and Tolerability of Ivermectin and Albendazole Mass Drug Administration in Lymphatic Filariasis Endemic Communities of Tanzania: A Cohort Event Monitoring Study. Pharmaceuticals (Basel). 2022 May 12;15(5):594. doi: 10.3390/ph15050594. PMID: 35631420; PMCID: PMC9147720.

III. **Fimbo AM**, Rajabu Hussein Mnkugwe, Eulambius Mathias Mlugu, Peter Kunambi, Bruno P. Mmbando, Omary MS Minzi, Appolinary A.R. Kamuhabwa and Eleni Aklillu, Surveillance of haematological and biochemical changes following mass Ivermectin and Albendazole administration for the control of lymphatic filariasis in endemic communities of Tanzania. *(Manuscript submitted to Infectious Diseases of Poverty)***.**

IV. **Fimbo AM,** Mlugu EM, Kitabi EN, Kulwa GS, Iwodyah MA, Mnkugwe RH, Kunambi PP, Malishee A, Kamuhabwa AAR, Minzi OM, Aklillu E. Population pharmacokinetics of ivermectin after mass drug administration in lymphatic filariasis endemic communities of Tanzania. CPT Pharmacometrics Syst Pharmacol. 2023 Dec;12(12):1884-1896. doi: 10.1002/psp4.13038. Epub 2023 Sep 11. PMID: 37638539; PMCID: PMC10725270

V. **Fimbo AM,** Rajabu Hussein Mnkugwe, Eulambius Mathias Mlugu, Peter P. Kunambi, Bruno P. Mmbando, Omary MS Minzi, Appolinary A. R. Kamuhabwa and Eleni Aklillu. Efficacy of ivermectin and albendazole combination in suppressing transmission of lymphatic filariasis following mass administration in Tanzania: A Prospective Cohort Study *(Manuscript)*.

Contents

List of abbreviations

ABA	Abamectin
ADL	Activities of Daily Living
ADR	Adverse Drug Reaction
AE	Adverse Event
ALB	Albendazole
ALP	Alkaline Phosphatase
ALT	Alanine-Amino-Transferase
AST	Aspartate aminotransferase
AUC	Area Under the Concentration Time Curve
BBB	Blood-Brain Barrier
BilD	Direct Bilirubin
BilT	Total Bilirubin
BMI	Body Mass Index
CBC	Complete Blood Count
CDC	Centers for Disease Control and Prevention
CDDs	Community Drug Distributors
CEM	Cohort Event Monitoring
CFA	Circulating Filarial Antigen
CL	Clearance
Cmax	Concentration Maximum
CNS	Central Nervous System
COVID	Corona Virus Disease
CRET	Creatinine
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
CV	Coefficient of Variation
CYP	Cytochrome P

DA	Diethylcarbamazine and Albendazole
DALYs	Disability Adjusted-Life Years
DBS	Dried Blood Spots
DEC	Diethylcarbamazine
DILI	Drug induced liver injury
DNA	Deoxyribo-Nucleic Acid
DOT	Directly Observed Therapy
EDCTP	European and Developing Countries Clinical Trials Partnership
EDN	Eosinophil Derived Neurotoxin
EDTA	Ethylene Diamine Tetraacetic Acid
EHC	Entero-Hepatic Circulation
EMA	European Medicines Agency
EO	Eosinophils
ESI	Electrospray Ionization
F	Bioavailability parameter
FOCE-I	First-order Conditional Estimation with Interaction
FTS	Filarial Test Strip
GFR	Glomerular Filtration Rate
GIT	Gastrointestinal tract
GOF	Goodness of Fit
GPELF	Global Programme to Eliminate Lymphatic Filariasis
GSK	GlaxoSmithKline
Hb	Haemoglobin
HCT	Haematocrit
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
HQC	High Quality Control

IA	Ivermectin and Albendazole
ICSRs	Individual Case Safety Reports
IDA	Ivermectin, Diethylcarbamazine and Albendazole
IIV	Inter-Individual Variability
IL	Interleukin
IQR	Interquartile Range
IS	Internal Standard
Ka	Absorption Rate Constant
KI	Karolinska Institutet
LC	Liquid Chromatography
LC-MSMS	Liquid Chromatography tandem Mass Spectrometry
LF	Lymphatic Filariasis
LLOQ	Lower Limit of Quantification
LQC	Low Quality Control
LYMPH	Lymphocytes
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
MDA	Mass Drug Administration
Mf	Microfilariae
MoH	Ministry of Health
MONO	Monocytes
MPV	Mean Platelet Volume
MQC	Middle Quality Control
MRCC	Medical Research Coordinating Committee
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry
MSD	Merck Sharpe and Dohme

MTT	Mean Transit Time
MUHAS	Muhimbili University of Health and Allied Sciences
NEUT	Neutrophils
NIMR	National Institute for Medical Research
NMRAs	National Medicines Regulatory Authorities
NN	Number of Transit Compartments
NONMEM	Nonlinear Mixed Effects Modelling
NPELF	National Programme for Elimination of Lymphatic Filariasis
NTDCP	Neglected Tropical Diseases Control Programme
NTDs	Neglected Tropical Diseases
ODK	Open-source Data Kit
OFV	Objective Function Value
Oncho	Onchocerciasis
OR	Odds Ratio
PCT	Plateletcrit
PcVPC	Prediction-corrected Visual Predictive Check
PDW	Platelet Distribution Width
PG	Pharmacogenetics
Pgp	P-glycoprotein
PhD	Doctor of Philosophy
PK	Pharmacokinetics
PLCR	Platelet Large Cell Ratio
PLT	Platelets
PMS	Post Marketing Surveillance
POPPK	Population Pharmacokinetics
PROFORMA	Pharmacovigilance infrastructure and post-marketing surveillance system capacity building for regional medicine regulatory harmonization in East Africa

PV	Pharmacovigilance
Q	Inter-compartment Clearance
QC	Quality Control
RBC	Red Blood Cell
RR	Relative Risk
RSE	Relative Standard Error
SAE	Serious Adverse Event
SD	Standard Deviation
SIDA	Swedish International Development Agency
SmPC	Summary of Product Characteristics
SSA	Sub-Saharan Africa
STH	Soil-Transmitted Helminths
TAS	Transmission Assessment Survey
TB	Tuberculosis
TFDA	Tanzania Food and Drugs Authority
Tmax	Maximum Time
TMDA	Tanzania Medicines and Medical Devices Authority
TNF	Tumor Necrosis factor
TPE	Tropical Pulmonary Eosinophilia
UK	United Kingdom
USA	United States of America
Vc	Central Volume
Vp	Peripheral Volume
VPC	Visual Predictive Check
WBC	White Blood Cell
WHA	World Health Assembly
WHO	World Health Organization

Introduction

Pharmacovigilance (PV) is one of the critical regulatory functions that allow for adverse drug events or reactions to be detected, analysed, and prevented. It is part of the post-marketing surveillance (PMS) method that ensures drugs are monitored for their safety soon after marketing authorization. Regulatory authorities are responsible for this role, and they are required to set up robust systems that work efficiently and effectively to protect humans from using drugs that are unsafe for consumption. The system is the gold standard in assessing the safety and efficacy of drugs once introduced in clinical practice. This PhD project applied the principles of PV to assess the safety and efficacy of drugs used during mass drug administration (MDA) campaigns in lymphatic filariasis (LF) endemic communities of the north-eastern part of Tanzania. Drugs used for LF during MDA include ivermectin and albendazole (IA). MDA is usually organized on an annual basis by the Neglected Tropical Diseases (NTD) control programme and during such campaigns, IA combination is always distributed at random to at-risk populations without any prior diagnosis of diseases. Whether individuals exposed have any underlying clinical condition or not, is not under consideration by the NTD programme. Exposing individuals of different age categories to drugs can potentially cause harm and therefore the need to monitor their safety.

This PhD project began by conducting a prevalence study to determine individuals harbouring worms in their blood. It is known that high levels of parasites including dead worms can cause inflammatory response and consequently development of AEs. The prevalence study allowed for the screening of a cohort of individuals to identify those with circulating filarial antigens (CFA) who were later checked for microfilaremia and then followed up for assessment of the efficacy of the drugs. The efficacy of MDA drugs was also put under the spotlight because since when MDA was introduced in Tanzania in 2002, LF has not been eliminated.

To be more comprehensive, the project further examined individuals for haematological and biochemical parameters to verify if they change soon after

drug administration. Observing and recording any change in these parameters is key in determining the safety of drugs and this is even critical when vital organs including the kidneys and liver are involved.

Since pharmacokinetics (PK) of drugs can influence the safety and efficacy of drugs, this PhD project further assessed the disposition kinetics of ivermectin (IVM). Despite that IA is given in combination during MDA, Ivermectin (IVM) only was chosen for PK analysis. This is because IVM has a dosage regimen that is unique as it considers the weight of an individual. We wanted also to explore whether individuals receive the correct dose of the drug since height poles are normally used to estimate the weight and therefore dose of IVM during MDA.

Taking the context into perspective and considering the nature of the project, five studies were therefore conducted as delineated in this thesis. The methods, results, and conclusions of each study have also been outlined in detail.

1 Literature review

1.1 An Overview and Global Burden of Neglected Tropical Diseases

Neglected Tropical Diseases (NTDs) are diseases of poor settings which have been forgotten. Many countries including national disease surveillance programmes have not taken deliberate measures or efforts to prevent or eliminate them. They are usually endemic in areas with tropical and subtropical climates (high temperatures and humidity) and where people do not have access to clean water (poor sanitation), are in close contact with vectors, unsafe ways to dispose of human waste, and inadequate healthcare delivery services [1, 2]. The majority of these diseases are found in Africa, Asia, and Latin America [3, 4]. The diseases have likewise received low attention on national and international health agendas [1].

NTDs include Buruli ulcer, Chagas disease (American trypanosomiasis), dengue and chikungunya, dracunculiasis (Guinea worm disease), echinococcosis, foodborne trematode infections (clonorchiasis, fascioliasis, opisthorchiasis, and paragonimiasis), human African trypanosomiasis (African Sleeping Sickness), leishmaniasis, leprosy (Hansen's disease), lymphatic filariasis (LF) (elephantiasis), mycetoma, onchocerciasis (Oncho) (river blindness), rabies, scabies, schistosomiasis, loiasis (African or Tropical eye worm), soil-transmitted helminths (STH) infections (Ascaris, Hookworm, and Whipworm), taeniasis and cysticercosis, trachoma and yaws (endemic treponematoses) [4-6].

The diseases are still a global burden to many countries and are responsible for many illnesses, suffering, stigma, and low productivity, particularly in impoverished communities [7, 8]. Most families are to-date trapped in a cycle of diseases and poverty [6]. Overall, NTDs affect approximately 2 billion people globally including 0.5 billion children, and are responsible for causing around 200,000 deaths annually [6]. Herricks *et al.* published a review study on the global burden of diseases in 2017 and identified soil-transmitted helminthiases, schistosomiasis, and food-borne trematodiases as the most common NTDs worldwide. On the other hand, visceral leishmaniasis, rabies, and Chagas disease were recognized as the most common causes of death related to NTDs globally [9]. These diseases together represent a loss of 25.1 million disability-adjusted life years (DALYs), 16.9 million years of those with disability, and 8.21 million years of life lost [9].

Amongst the NTDs, the following can be controlled or even eliminated through mass drug administration (MDA) or other effective interventions – Dracunculiasis, LF, Oncho, Schistosomiasis, STH, and Trachoma [4]. However, with resource constraints and low socioeconomic status amongst communities, these diseases are still endemic. Well-designed and regular studies need to be done to determine

the prevalence and disease extent for the long-term elimination of NTDs to free communities from such blight.

1.2 The Global Burden of Lymphatic Filariasis

Lymphatic filariasis (LF) is one of the NTDs that still affects endemic communities. It is also endemic in Sub-Saharan Africa (SSA) affecting all age groups including children [3, 5]. In 1996, WHO estimated that some 120 million people worldwide were affected by LF, of whom about 40 million were incapacitated and disfigured by the scourge [5, 10]. In 2000, about 40% of LF-infected people were from SSA, with cases ranging from 46 to 51 million, and an estimated at-risk population of 432 million people [5, 10, 11]. By 2015 it was estimated that about 251 million people were living in areas of LF transmission in SSA [5, 12]. Of these, 96 million lived in areas co-endemic with both LF and Oncho and 83 million in LF mono-endemic areas [5, 12]. In 2018, the total estimated at-risk population requiring intervention in Africa was 341 million [13]. Again, in 2018, 893 million people in 49 countries were living in areas that required preventive chemotherapy to stop the spread of LF [10]. Between 2000 and 2020, 8.6 billion cumulative treatments were distributed to more than 925 million people at least once in 68 countries [14].

The disease is usually acquired during childhood with visible manifestations observed later in life [10, 15-17] . It is the leading cause of long-term disability worldwide causing lymphoedema (elephantiasis), hydrocele, and adenolymphangitis [5, 15, 18]. In 2000, around 15 million people were estimated to have lymphoedema and 25 million men had urogenital swelling, principally scrotal hydrocele [5, 13]. The WHO estimated approximately 40 million individuals suffered from the stigmatizing and disabling clinical manifestations of the disease in 2000 [4, 5, 13].

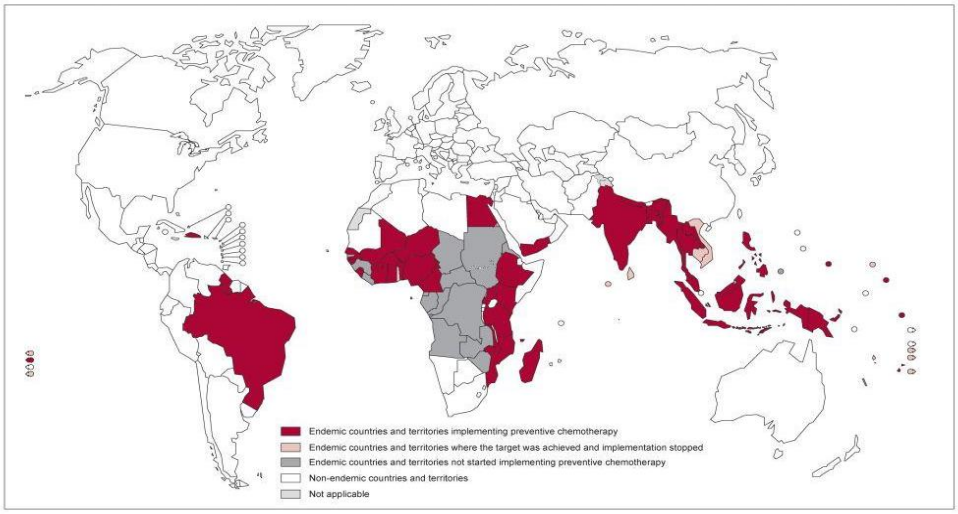


Figure 1: *LF endemic and non-endemic countries. Source: [19].*

1.3 Global Programme to Eliminate LF

Many efforts are currently being undertaken by countries to prevent and eliminate LF. Controlling the vectors (i.e., mosquitoes) that transmit the disease and improving sanitation and hygiene are highly effective strategies against LF [4, 5]. The 50th World Health Assembly (WHA) meeting which was held in 1997 resolved to eliminate LF as a public health problem (Resolution WHA 50.29) [5]. The Global Programme to Eliminate Lymphatic Filariasis (GPELF) was then launched by WHO in 2000 [5]. The overall goal of GPELF was to eliminate LF as a public health plague by 2020 [5, 13]. Two strategies were deployed for countries to adopt - MDA of microfilaricidal drugs to the entire at-risk population to halt transmission and morbidity management in case of those who have full-blown disease [20, 21].

MDA is effective in reducing infection to levels below the threshold at which mosquitoes cannot continue to spread the parasites from one person to another and therefore prevent transmission of infection. Depending on endemicity and the type of disease prevailing in the concerned community, the WHO recommends the use of two drug regimens – a combination of Ivermectin and Albendazole (IA) or Ivermectin, Diethylcarbamazine and Albendazole (IDA) [5]. IA is used in areas where LF is mono-endemic and is not recommended in areas where co-endemicity with Oncho or loiasis exists [5]. This is due to safety concerns as the IDA combination is associated with life-threatening adverse drug reactions (i.e., Mazzotti reactions) in those exposed [5].

Since the introduction of GPELF, regular studies and field research need to be done to measure the prevalence of LF and interventions targeting the reduction of disease transmission. This will also ascertain the impact of long-term MDA to

at-risk populations and its effectiveness in reducing morbidity and disease burden to communities. The programme itself needs to be evaluated to figure out if it attains its envisaged milestones or not.

1.4 The Burden of LF in Tanzania

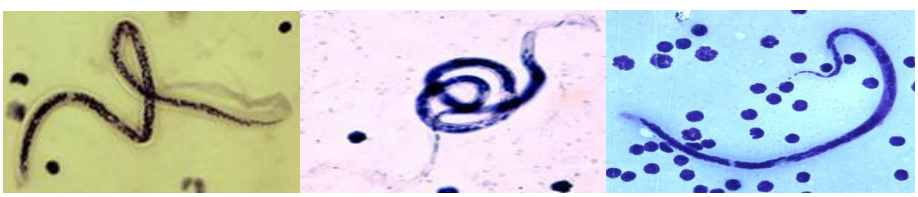
Tanzania has not been spared by LF. The country ranks third of the African nations in terms of the highest burden of LF, with over 34 million people at risk of acquiring the disease, and more than 6 million infected [8, 22, 23]. The disease is more prevalent along the coastline of the Indian Ocean where Mkinga district is also located. Men are the most affected as they most of the time spend their time fishing on the offshores of the Indian Ocean. At the time of MDA, they are the ones who also miss out on drug administration due to this sort of lifestyle.

Mosquito vectors are responsible for the wide spreading of the disease. The transmission of LF is through mosquito bites and the disease follows the malaria transmission pattern. Nevertheless, patterns of infection have also been observed in the inner parts of mainland Tanzania [23-25]. Vector control using insecticide-treated nets, residual spraying, and avoiding mosquito bites, particularly during the night, plays a crucial role in LF control.

Poor MDA coverage has also proven to be a factor in the ongoing transmission of the disease. MDA campaigns conducted in endemic districts of the country with good coverage can subsequently reduce disease transmission and enable the country to achieve LF elimination. Post-MDA surveillance and Transmission Assessment Surveys (TAS) after at least 5 consecutive MDA campaigns are recommended by WHO [5]. Such a survey must measure that transmission is declining to levels where recrudescence is unlikely to occur.

1.5 Life Cycle and Transmission of LF

LF is transmitted by three species of thread-like nematode worms namely – *Wuchereria bancrofti, Brugia malayi, and Brugia timori* [5, 26]. Male worms are about 3–4 centimeters in length, and female worms 8–10 centimeters [16].



Wuchereria bancrofti ***Brugia malayi*** ***Brugia timori***

Figure 2: *Nematodes responsible for transmission of LF.* Source: [19].

W. bancrofti is the most common and accounts for many disease conditions (>90%) seen in patients [26, 27]. The life cycle of *W. bancrofti* involves two hosts

(i.e., humans and mosquitoes). The adult parasites that reside in the lymphatics of the human host produce first-stage larvae (microfilariae - mf) after mating which move to the peripheral blood circulation. The mf exhibits diurnal periodicity and normally resides in the deep vein during the day before migrating to the peripheral circulation during the night. They reach peak concentrations in 4 hours from around 10 pm to 4 am. This occurs due to an adaptation of the worms to the biting behaviours of the vector (i.e., mosquitoes) responsible for the transmission of LF [28]. During the feeding episode of these vector mosquitoes, mf is taken up during the night from the infected host and matures into the motile larvae which are then transmitted to a new human host during the next feeding. All mosquito species including Culex, Aedes, and Anopheles are responsible for transmission of LF [5, 26]. The mature larvae then migrate to the bloodstream and move through the lymphatic system to regional lymph nodes, predominantly in the legs and genital areas [29] where it is responsible for several clinical manifestations [30]. The larvae then develop into adult worms over one year and reach sexual maturity in the afferent lymphatic vessels. After mating, the adult female worm can produce thousands of mf that migrate to the peripheral bloodstream to repeat the life cycle [10]. The worms can live for approximately 6–8 years during their lifetime, producing millions of mf circulating in blood [30].

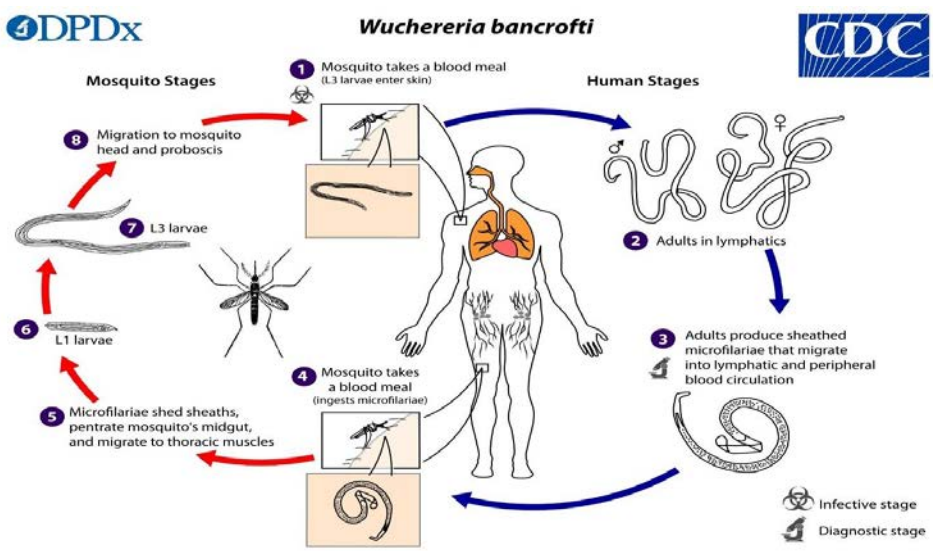


Figure 3: *Life cycle of LF. Source:* [31].

1.6 Clinical Manifestations of LF

LF infection involves asymptomatic, acute, and chronic stages. Despite that all age groups can be affected transmission is usually during early childhood with consequences observed later in life [11, 16, 23]. The male and female worms together form "nests" in the human lymphatic system - the network of nodes and vessels that maintain the delicate fluid balance between blood and body tissues [32]. The majority of infections are asymptomatic, without any external signs of infection contributing to transmission of the parasite. These asymptomatic infections still cause damage to the lymphatic system and the kidneys and alter the body's immune system. The kidney damage can be as many as 40% with proteinuria and haematuria [16, 33]. When LF develops into a chronic condition it leads to lymphoedema (tissue swelling) or elephantiasis (skin/tissue thickening) of limbs and hydrocele (scrotal swelling). Involvement of breasts and genital organs is also common [14].

Acute episodes of local inflammation involving skin, lymph nodes, and lymphatic vessels often accompany chronic lymphoedema or elephantiasis. Some of these episodes are caused by the body's immune response to the parasite. Most are the result of secondary bacterial skin infections where normal defenses have been partially lost due to underlying lymphatic damage. These acute including recurrent attacks are painful and often accompanied by fever [5, 14]. They may also last for weeks and are the primary cause of lost wages amongst people suffering from LF. Lymphoedema and hydrocele are the classic clinical manifestations taken into account by NTD programmes when measuring disease prevalence and morbidity in communities.

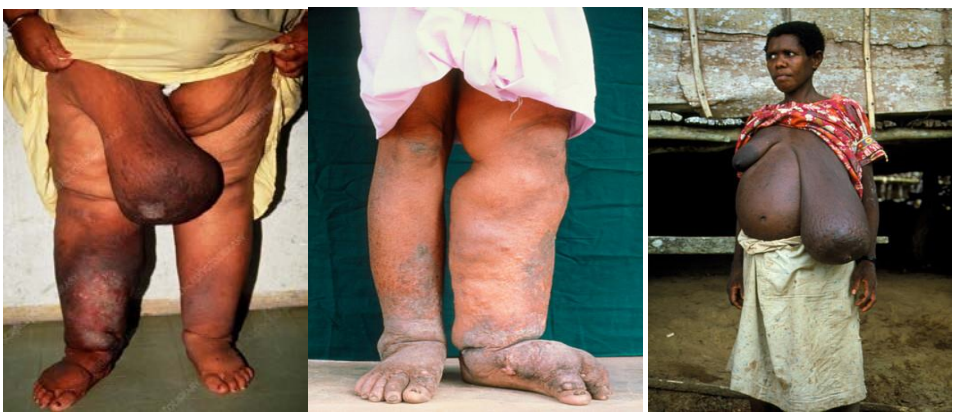


Figure 4: *Clinical manifestations of LF. Source: [34].*

1.7 Diagnosis of LF

Diagnosis of LF is two-fold. The first stage involves the detection of antigens in the blood of an infected individual. This involves screening of individuals for CFA using the Filariasis Test Strip (FTS). The FTS detects CFA released in the blood by adult worms [35]. There are few manufacturers of these strips including Alere©, Waltham, United States. Briefly, the test involves, a finger prick to be made using a sterile disposable lancet to collect blood samples. A special pipette may be used to apply approximately 75 µL of blood on the FTS and reading results in a few minutes (e.g., 10 min).

After FTS, all antigenemia-positive individuals are eligible for determination of mf in blood. Such individuals should be identified and approached for night blood sampling. Finger prick blood samples should be taken from individuals at night between 9 pm to 4 am and poured onto 75 µL capillary tubes. Each blood sample is dispensed into a 1.8 mL cryo-tube containing 900 µL of 3% acetic acid, mixed thoroughly, and transferred into the Sedgwick-Rafter counting chamber. Estimation of worms is henceforth done by counting the number of mf in the chamber, using a compound light microscope set at 4×magnification. Results are reported as the number of microfilariae per 75 µL of blood.

Figure 5: *FTS and Sedgwick-Rafter counting chamber showing detected microfilariae. Source: Test results obtained during laboratory investigations at NIMR Tanga.*

1.8 Prevalence of LF in Tanzania

Tanzania is amongst the SSA countries still affected by LF. An estimated 34 million people were at risk and six million were affected by the year 2000 [36, 37]. It was during this time that the National Lymphatic Filariasis Elimination Programme (NLFEP) was established.

The prevalence of mf and CFA in the community was 24.5% and 53.3% respectively, before MDA intervention in 2002 [22]. The same considerably

decreased after six rounds of MDA to 2.7% and 19.6%, respectively [22]. In 2004, the mf prevalence in the Tanga region before MDA was 24.5%, while that of CFA was 63.3%, and for specific antibodies to recombinant filarial antigen was 78.9% [38]. After 8 rounds of MDA, the CFA and mf prevalence in combined study communities was reduced by 75.5% and 89.6%, respectively, compared to baseline levels [39]. At the same time, the CFA prevalence in school children was reduced by 90.9% compared to baseline [38, 39].

In the same location, a reduction of infection prevalence to low levels of 1% was noted, but along the coast of the Indian Ocean, the prevalence of infection was still higher than what was expected [38, 40]. In the current PhD programme, the overall prevalence of CFA positivity at the end of 2018 in the Mkinga district, was 5.8% and that of mf was 5.2% [10]. Global and national efforts have been undertaken but still, LF continues to affect poor people especially those living in rural areas. As we have surpassed the WHO-GPELF target of 2020, evidence still suggests that the disease is still prevalent, and the elimination target has not been met yet.

1.9 Preventive Chemotherapy Strategies Through MDA Campaigns

Preventive chemotherapy is a disease control strategy that is used in high-endemic areas to reduce transmission or eliminate the disease from the community altogether. This can be achieved through administering medicines to the entire eligible population of an area at regular intervals, irrespective of the individual infection status (MDA). MDA has proven to play a key role in decreasing and reducing the transmission rates in populations at risk. It can also prevent the progression from sub-clinical to clinical stages of the disease including worsening morbidity. Drugs used for MDA as recommended by WHO in LF endemic communities include a combination of ivermectin and albendazole (IA) in areas not co-endemic with oncho or loiasis [5, 41].

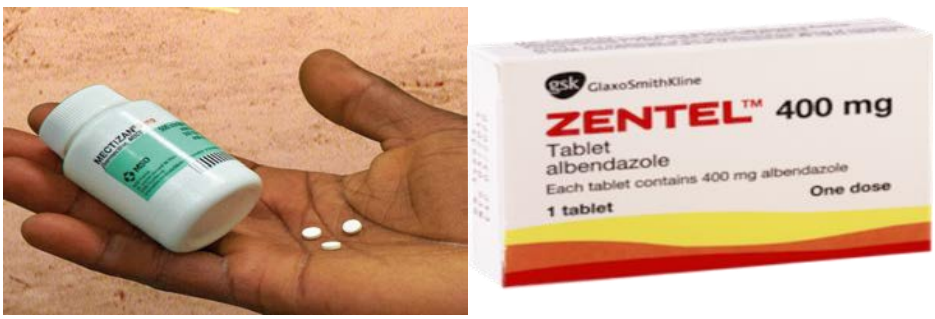


Figure 6: *Ivermectin and Albendazole tablets in their original packs. Source: Photos taken during fieldwork.*

These drugs are given as donations through the Mectizan Donation Programme [42] and administered on an annual basis at doses of ivermectin (150–200 μg/kg) with albendazole (400 mg) for the duration of the reproductive lifespan of adult parasites [5]. The drugs have a microfilaricidal effect and act by destroying the microfilariae but not adult worms [17]. The entire population at risk can be given such drug regimen but not pregnant women or children of less than 90 cm in height (approximately equivalent to < 15 kg body weight) and the severely ill [5]. The dose of IVM needs to be given based on the weight of an individual [21] as highlighted in **Table 1a.**

Table 1a: Weight-based dosing schedule for IVM during MDA

Weight range (kg)	Number of Tablets (mg)
15 - 22	1 (3mg)
23 - 37	2 (6mg)
38 – 52	3 (9mg)
53 - 67	4 (12mg)
68 - 82	5 (15mg)
83 - 97	6 (18mg)
≥ 98	7 (21mg)

However, due to logistical challenges including obtaining accurate weight measurements in remote and resource-limited areas as well as the feasibility of supplying adequate weighing scales during MDA, height-based dosing is currently recommended as an alternative to weight-based dosing during MDA [43, 44]. **Table 1b** below highlights the height-based dosing of IVM used by the NTD programme in Tanzania.

Table 1b: Height-based dosing schedule for IVM during MDA

Height (cm)	Number of Tablets (mg)
< 90	0
90 - 119	1 (3mg)
120 - 139	2 (6mg)
140 – 159	3 (9mg)
> 159	4 (12mg)

Source: [45]

The effectiveness of the MDA strategy depends on epidemiological coverage, which is defined as the proportion of the total population ingesting the medicines during MDA [5]. WHO considers at least 65% epidemiological coverage to be an effective MDA round. In programmes where drug coverage is poor or where

transmission is particularly intense, more than five MDA rounds are needed to lower levels of infection below elimination thresholds [5].

1.10 Safety Surveillance Through Cohort Event Monitoring (CEM) Method

Safety surveillance of medicines begins during pre- or non-clinical stages. This early phase is pivotal for protecting the health of individuals taking part in clinical drug development [46]. The same needs to be expanded to detect and minimize risks to drug users after marketing authorization. Upon approval of a medicinal product, what follows is post-marketing surveillance (PMS) which also embraces the pharmacovigilance (PV) of marketed products. The WHO defines pharmacovigilance *"as the science and activities relating to the detection, assessment, understanding, and prevention of adverse effects or any other medicine/vaccine-related problem"* [47, 48].

Two methods of safety data collection are deployed in PV programmes by many countries. These include spontaneous or passive and active reporting methods. Passive reporting involves the voluntary sending of reports by healthcare providers or patients to National Medicines Regulatory Authorities (NMRAs). This method has experienced mounting challenges including under-reporting of AEs. Experience shows that few reports are received by NMRAs and the factors associated are well reported [49-52].

On the flip side, many authorities have now switched to the active safety reporting method which has proven to be instrumental, particularly in increasing the number of AE reports. Active safety surveillance involves the systematic collection of information on the presence or absence of AEs within a defined group of people [53]. One type of active safety surveillance method that has been piloted and is now widely used is cohort event monitoring (CEM). CEM uses intensive measures of collecting data on AEs that may occur as a result of drug use in a particular setting [54-56]. Compared to a passive reporting system, CEM has many advantages in PV as the method is capable of providing incidence rates of AEs, characterizing known ADRs, detecting interactions with other medicines, identifying risk factors, and providing a measure of comparative risk between medicines [54-57]. With the CEM method, proactive measures are taken to ask for events from individuals or patients, and the same is applied within a short time [54-56]. This method can be deployed to monitor the safety of medicines used for diseases of public health importance or for new medicines and public health interventions that have recently been introduced in clinical practice. The WHO also recommends a seven-day interval of safety monitoring of drugs used in public health interventions using this method [47]. Tanzania began using the CEM method in 2016 which involved a follow-up of an anti-malarial combination drug

namely artemether + lumefantrine (ALu) [58]. Since then, experience in organizing and conducting such studies has been gained and the method was adopted in conducting the safety study reported in this current doctorate programme.

1.11 Safety of Ivermectin and Albendazole

Ivermectin and albendazole are the drugs of choice for MDA in LF mono-endemic communities. Combinations containing diethylcarbamazine (DEC) are also used in other countries but not recommended in areas where there is co-endemicity between LF and oncho or loiasis. This is due to severe adverse drug reactions (Mazzotti reactions characterized by pruritus, rash, fever, malaise, lymphadenopathy, arthralgia, tachycardia, hypotension, oedema and abdominal pain) observed and documented [59-62].

Ivermectin acts by killing the larvae stage of mf but not adult worms [17] and it binds with high affinity to glutamate-gated chloride channels which occurs in the invertebrate nerve and muscle cells, causing an increase in the permeability of the cell membrane to chloride ions with hyperpolarization of the nerve or muscle cell [59, 63, 64]. Albendazole has a vermicidal effect and acts by causing degenerative alterations in the intestinal cells of the worm by binding to the colchicine-sensitive site of β-tubulin of parasites, thus inhibiting its polymerization or assembly into microtubules [65]. Taking into account their modes of action, both drugs may be associated with AEs. The inflammatory response that arises due to the presence or death of parasites in the lymphatic or blood streams has been found to trigger and elicit AEs. Mild to moderate systemic AEs related to the death of mf were also reported by Budge *et. al*. in their review of the literature [66]. Severe ADRs have been observed in patients with more than 30,000 microfilariae/ml and the risk is very high with loads above 50,000 microfilariae/ml [67, 68]. Many other studies have also reported an association between the chronic manifestation of LF as a risk factor and the development of AEs after using a combination of IA [66, 69-72]. Central and peripheral nervous system disorders associated with IVM use were also reported by Nzolo *et.al.* in the search of Vigibase in 2017 [73].

Commonly known AEs associated with ivermectin include nausea, diarrhoea, dizziness, itching, skin rash, swelling of the hands or feet, swelling or tenderness of lymph nodes, weakness, confusion, lack of coordination or vertigo, somnolence, urinary or bowel problems, seizures or convulsions, eye redness and vision changes or problems with vision [10, 69]. These ivermectin-induced AEs have been observed during non-clinical and clinical investigations and documented in the summary of product characteristics (SmPC) and package inserts published by manufacturers [74]. Almost similar events had also been reported for albendazole alone [75]. PV of IA during MDA in Tanzania had not been practised before and this has been the centre stage of the current doctorate programme to

comprehensively investigate the safety of the concerned drugs after mass community exposure.

1.12 Need for Safety Surveillance During MDA

During MDA, drugs are administered on a large scale to at-risk populations without prior screening or diagnosis. Communities are highly exposed to preventive chemotherapy and the PV infrastructure is weak. Given the relatively poor systems and limited resources in these settings, PV data collected is scarce, particularly in SSA. Safety surveillance studies to detect the types, severity and risk factors of AEs during MDA are vital for the detection, assessment, understanding and prevention of adverse effects. Occurrence of AEs following MDA can cause non-adherence to MDA, which may profoundly impact the treated community and jeopardize programme success. Communities which are well-informed about the type and severity of AEs to be expected may be less likely to avoid MDA out of fear of AEs, therefore leading to increased public confidence.

PV is a key function of any medicine's regulatory authority, Tanzania inclusive. In Tanzania, the Tanzania Medicines and Medical Devices Authority (TMDA) is mandated by law (*The Tanzania Medicines and Medical Devices Act, Cap 219*) [76] to regulate the quality, safety and efficacy of medicines. The TMDA has set up a robust regulatory mechanism to control the manufacturing, importation, distribution, supply and sale of medicines in mainland Tanzania. Through its PV system, the TMDA can detect AEs that occur in those exposed to medicines in the country, analyse them and take regulatory action(s). The PV system was first introduced in 1993. However, the system still experiences challenges including under-reporting of AEs from healthcare providers or patients. PV had also not been very well integrated into the NTD programme to collaborate in the reporting of AEs for drugs used in this PHP.

Taking these into account and since safety monitoring had not been very active since the introduction of MDA in 2002, the reported safety study was therefore conducted adopting a CEM methodology. The study recruited a large sample size as previous reports indicate that other studies conducted elsewhere were of few sample sizes and most of them were inconclusive on the safety of IA.

1.13 Monitoring of Biological Parameters Following MDA

Monitoring of changes in biological parameters after drug use is not very common in SSA. The same is also true for drugs used in MDA programmes. Many investigators follow up safety of medicines by only collecting data on AEs reported but not conducting laboratory investigations to figure out any changes in haematological and biochemical parameters. The reason could be it is costly and time-consuming to do so. Nonetheless, for adequate investigation of the

safety of drugs, it is pivotal to design and organize safety studies targeting the measurement of biological parameters. This would allow for a comprehensive assessment of drug-related illnesses particularly involving the vital organs such as the kidneys, liver and heart. It would also help in establishing the mechanism of preventing drug-induced AEs and safeguarding public health. Many studies on haematological and biochemical changes after IVM and ALB use have been explored in animals and reported [77-83]. Few studies conducted in humans have also reported haematological and biochemical changes after the use of IVM or ALB alone or in combination despite their small sample sizes and inconclusive findings. Most of these studies involving IVM alone focused mainly on the use of the drug in onchocerciasis.

IA are both metabolized in the liver through the cytochrome P450 enzyme system [33, 84]. Both have the potential to cause liver injury. The first case of IVM-induced severe liver disease was reported on a 20-year-old woman originally from Cameroon one month after using a single dose of the drug in 2006 [85]. Hepatic and renal disorders after IVM administration were also reported by Campillo *et.al* in the review of Vigibase, the WHO's global individual case safety reports database [59]. Significant changes in kidney function were observed in a study done in Cameroon whereby serum creatinine and urea levels were significantly altered after IVM use [86]. In this same study, however, the liver function was normal after repeated administration of IVM as AST and ALT levels did not change. A study done in Ghana and Ecuador demonstrated that treatment of patients with onchocerciasis with high-dose of IVM was associated with marked post-treatment increases in plasma tryptase levels and local reactions were strongly correlated with eosinophil derived neurotoxin (EDN) and interleukin-5 (IL-5) [87]. A clear correlation between the appearance of mast cells and attacks on damaged mf by eosinophils and macrophages and in adult worms by neutrophils after IVM treatment was likewise observed in a study done by Wildenburg *et. al.* [88]. Furthermore, Zheng et. al. compared the effect of IVM with that of DEC on several parameters of immune response and concluded that both treatments triggered an increase in serum levels of IL-1, TNF and IL-6 in all patients studied [89]. No elevated TNF alpha levels were found, but IL-6 and CRP were elevated in patients after IVM treatment in a study done by Njoo *et al* [90]. In a study done by Burchard *et.al.*, a slight but significant increase in the excretion of urinary albumin and alpha1-microglobulin was seen five days after IVM treatment in all treated patients, whereas levels of proteinuria were significantly higher only in patients with high microfilarial densities [91]. The study concluded that IVM can cause minor and not clinically relevant glomerular and tubular disturbances in patients.

For a comprehensive safety evaluation, this doctorate programme thoroughly investigated the haematological and biochemical parameters in CFA-positive and

negative individuals to find out any changes in parameters as a measure of the safety of IA following MDA.

1.14 Pharmacokinetics Analysis of MDA Drugs

IA combination preventive chemotherapy has been deployed during MDA on an annual basis in Tanzania since 2002. The effectiveness of these drugs during MDA depends in part on their PK disposition. Differences in anatomical, physiological and biochemical characteristics amongst exposed individuals may influence PK profiles. Albeit, these combination drugs have been used since then, the PK disposition of IVM in particular has not been fully evaluated in different populations. The review of literature has highlighted that PK studies of IVM alone have been conducted mostly in a low number of healthy adults or adults infected with *Onchocerca volvulus* or *Plasmodium falciparum* malaria [62, 92-96]. A PK characterization of IVM is critical to determine its disposition when exposed to antigenemia-positive or negative individuals in LF-endemic communities. As the drug has been and will continue to be used in MDA, and since individuals with or without prior disease manifestations including LF, are annually exposed, there is a need to comprehensively study the disposition of IVM to consistently deploy it safely and effectively in future MDA campaigns.

As indicated above, IVM is metabolized in the liver through the cytochrome P450 enzyme system [33, 84]. The drug is highly lipophilic with high membrane permeability and its plasma exposure depends on the body mass index (BMI) of an individual [97]. Its protein bound is 93% and it does not cross the blood-brain barrier (BBB) [92, 98-100]. P-glycoprotein (Pgp) plays a role in its uptake into the central nervous system (CNS) causing neurotoxic effects such as ataxia, tremors, myoclonus, seizures, encephalopathy and coma as reported [101, 102]. Similarly, Pgp is also responsible for the efflux of IVM from the gastrointestinal tract (GIT) membrane affecting its bioavailability [101]. Its elimination half-life ranges between 25 and 80 hours [98, 99, 103]. The drug including its metabolites is excreted mainly in faeces and only 1% in urine as previously reported [104].

ALB is a substrate of CYP450, mainly through CYP2J2 and to a lesser extent through CYP3A4/5 [105]. Through this pathway, ALB by itself may compete with IVM for the same metabolism. All these characteristics may differ between populations and potentially affect the disposition of IVM and therefore the need for further evaluation. IVM showed a lower exposure profile in children compared with adults, in a study done by Schulz *et. al.* [96], highlighting the need to establish dosing recommendations for different age groups. Dosing is critical in MDA as IVM is always given based on body weight.

Despite its widespread use, the PK characteristics of IVM are still poorly understood. Great awareness of its characteristics will improve clinical efficacy,

especially in future MDA programmes. This had therefore been the centre stage of this PhD programme to evaluate the PK disposition of IVM when deployed during MDA in endemic communities of north-eastern Tanzania.

1.15 Population Pharmacokinetics Modelling

The PK sub-study in this PhD programme focused on blood sample collection from individuals taking part in MDA in rural communities. The timing of drug administration and consequently sampling for PK analysis was different to each individual depending on their availability and once they show up at the study sites identified. The elimination half-life of IVM ranges between 25 and 80 hours [98, 99, 103]. This long half-life also helps to kill the mf stage of the adult worms once they migrate to the blood circulation from the lymphatic system during the night. Considering the long elimination half-life of the drug, it was not feasible to prolong blood sampling up to 80 hours with the sample size targeted.

Since population PK (POPPK) modelling can help overcome this challenge, it was therefore decided to adopt a modelling approach to analyse the PK parameters. POPPK modelling is a concept which had recently been introduced and it has been instrumental in helping researchers to describe PK disposition kinetics of drugs in populations. Modelling can be applied in case of missing PK data or in areas where it is not feasible to conduct PK studies, particularly for drugs with longer elimination half-lives. The model can utilize few sampling points, ensures adherence to study protocols and minimizes losses to follow-up. It can also be used to precisely estimate the expected or observed data from the population studied. Through modelling an estimation of the $AUC_{0-\infty}$ and elimination half-life which are critical PK parameters for determining drug exposure as a measure of bioavailability can easily be done. It can as well be used to test for any covariates that may predict or influence the PK exposure of a drug. The POPPK model was therefore adopted to estimate PK parameters from blood samples collected at four sampling points (i.e., pre-dose and at 2-, 4-, and 6-hours post-dose) in this PhD programme.

Different researchers have designed different models that are used by many in PK analysis. These are available and easily accessible from the literature. The oral route is the only approved for IVM administration in humans. The one and two-compartmental models with first-order oral absorption have been used to describe the IVM kinetic behaviour after its oral administration [106-108].

El-Tahtawy *et. al.* reported two prior population-based PK studies of IVM, both of which were re-analyses of interaction trials involving the drug. The first was a re-analysis of an interaction study with azithromycin using a two-compartment model with first-order oral absorption and elimination, and additionally implementing a mixture model for bioavailability [109]. Kobylinski *et. al.* [99] and

Na-Bangchang *et. al.* [110] reported a study in 2017 that employed a two-compartment model with linear elimination, with two transit absorption compartments. In this study, data was pooled from a trial involving healthy Thai volunteers with albendazole +/- praziquantel as a concomitant medication.

Duthaler *et. al.* developed a POPPK model to study the variability in PK of IVM focusing on body composition and enterohepatic circulation (EHC). In this study, dried blood spots (DBS) samples were used to relate the concentrations of the drug in plasma [111]. González Canga *et. al.* [104] and Chaccour *et. al.* [112] also reviewed the sources of PK variability including the proposed existence of EHC in their studies done previously.

Together with the models developed by El-Tahtawy *et. al.* [109] and Duthaler *et. al.* [111], our literature search in this PhD programme further identified three other POPPK models of IVM all of which reported 2-compartment disposition kinetics [113-115]. Based on comparisons of objective function values (OFV) obtained by Bayesian estimation of individual PK parameters using the identified models, we selected Duthaler's model [111] as the reference model for our modelling. In addition to its relatively lower OFV compared to other models, Duthaler's model of 2019 was based on rich PK samples, had good precision of parameter estimates, and its predicted mean concentration profiles were more comparable to our observed mean profiles than other models.

1.16 Efficacy of Ivermectin and Albendazole to Stop Transmission of LF

IA are deployed in combination during MDA to halt the transmission of LF. Prior evidence suggests that they are effective in killing the parasites responsible for the aetiology of LF. IVM alone exhibits a microfilaricidal effect and ALB alone has a vermicidal effect, as reported [17, 59, 63, 64] [65]. IVM cannot kill the adult parasites hence the need for its use in combination with ALB to exert the intended effect. Adult parasites nest in the lymphatic system and can live for an average of 6–8 years producing millions of mf [19, 116]. The same do not always die after treatment and MDA must be maintained for the duration of time the adult worms retain fecundity [5].

Many studies have been conducted before and reported the efficacy of IVM alone or in combination with ALB [117-125]. Nonetheless, the performance of the drug may be affected by many factors including drug exposure (Cmax and AUC), genetics, parasite stage (microfilaria versus adult worms) and resistance pattern. IA drug combination is capable of killing mf with minimal or no effect on adult filarial worms [5]. The fact that IA does not kill the adult worms, may contribute to the observed continued disease transmission in the endemic areas. The combination is further recommended for use in LF-endemic areas where the disease is not co-endemic with oncho and loiasis due to serious ADRs reported.

This has resulted in the avoidance of using combinations containing DEC (i.e., IDA or DA) in Tanzania which has proven to kill the adult parasites responsible for repeated transmission of the disease. The DA regimen is an effective microfilaricidal combination used for MDA in areas where oncho is not co-endemic. The medicines, used alone and in combination, have also shown macrofilaricidal effects against adult worms [126-129].

The development of a more efficacious, field-ready and safe macrofilaricide for LF has been sought, and research has been ongoing for several decades. Programmatic studies to delineate the absence of oncho and loiasis that demonstrate the safe introduction of DEC in countries are also needed. IDA combination has also an acceptable safety profile and is more effective for clearing mf. With adequate compliance and medical support to manage AEs, IDA has the potential to accelerate LF elimination in Tanzania. The same had been observed in a study done in India [130]. With persistent prevalence and continued exposure which might be attributed to the potential for parasite tolerance including the emergence of drug resistance, more studies on efficacy are warranted. The efficacy of drugs in reducing parasite reservoirs in the community and interruption of transmission is henceforth key to LF elimination.

2 Research aims

2.1 Broad Objective

The main aim of this PhD research project was to assess the safety and efficacy of drugs (i.e., ivermectin and albendazole) deployed during MDA in endemic communities of Tanzania. This was achieved first by determining the prevalence and correlates of LF infection followed by a CEM study that allowed for active safety follow-up of individuals in affected communities. The blood, liver, and kidney functions including the PK profile were further explored to characterize and determine their correlation with the safety and efficacy of ivermectin. Individuals exposed to IA were then followed up for six months to ascertain the efficacy of MDA drugs in suppressing infection.

2.2 Specific Objectives

1. To determine the prevalence and correlates of lymphatic filariasis infection and its morbidity following MDA in Mkinga District, North-Eastern Tanzania (**Paper I**).
2. To assess the safety of ivermectin and albendazole using cohort event monitoring method during MDA in lymphatic filariasis endemic communities of Mkinga district, North-Eastern Tanzania (**Paper II**).
3. To evaluate the haematological and biochemical changing patterns on surrogate markers after MDA in filariasis endemic communities of Tanzania (**Paper III**).
4. To evaluate ivermectin pharmacokinetic disposition including its predictors following MDA in Mkinga district, North-Eastern Tanzania (**Paper IV**).
5. To assess the efficacy of ivermectin and albendazole following MDA in suppressing transmission of lymphatic filariasis in endemic communities of rural Tanzania (**Paper V**).

3 Materials and methods

3.1 Study Setting

The fieldwork for this PhD research project was conducted in the Mkinga district, Tanga region – the north-eastern part of Tanzania (05°04'S, 39°06'E). Other districts in the Tanga region include Handeni, Kilindi, Korogwe, Lushoto, Muheza, Pangani, and Tanga. The district is situated along the coast of the Indian Ocean bordering Muheza district and Tanga city to the South, the Indian Ocean to the East, Korogwe and Lushoto districts to the West, and Kenya border to the North (**Figure 7**).

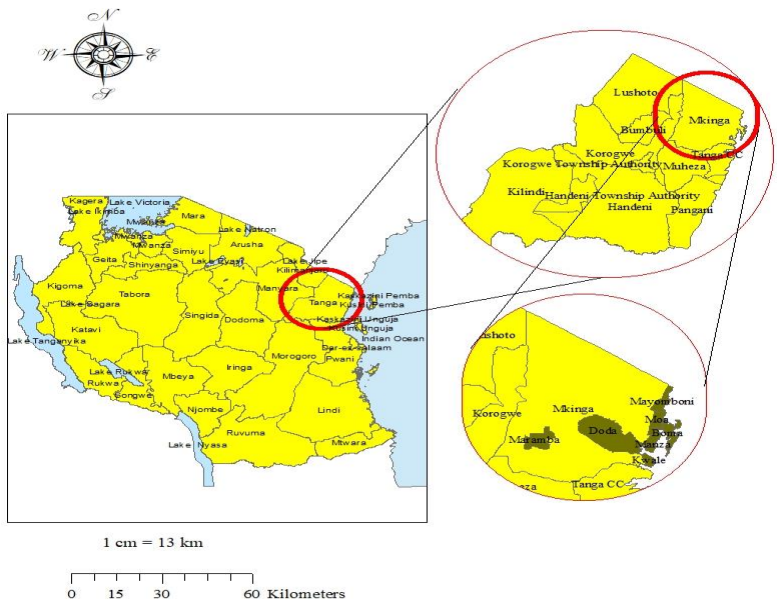


Figure 7: *Map of the study site. Left is the map of Tanzania located in the Eastern part of Africa. The top-right figure shows the map of the Tanga region, where the Mkinga district is located. The bottom-right figure shows the map of wards in the Mkinga district, whereby villages in these wards participated in this study. The study site map was originally generated using ArcGIS software version 10.7.1*

The district has two main rainy seasons in a year (bimodal): the long rains from March to June, and the less intensive short rains from November to December. The region has a warm and wet climate due to the influence of the Indian Ocean. Humidity is high and often goes up to 100% maximum and ranges from 65% to 70% minimum. According to the national census conducted in 2012, the Mkinga

district population was 118,065, with 48.9% (57,760) being male, and the population density was 44 people per sq. km. The altitude ranges from 0 to 1506 m above sea level, measuring from the Nilo peak [131]. The population of the Tanga region following 2022 census results was 2,615,597 [132]. The main economic activities in this district include fishing, subsistence farming, low-scale livestock keeping, and petty trading.

3.2 Study design, population, and sample size

Different study designs were adopted to attain the overall goal of the PhD project (**Figure 8**).

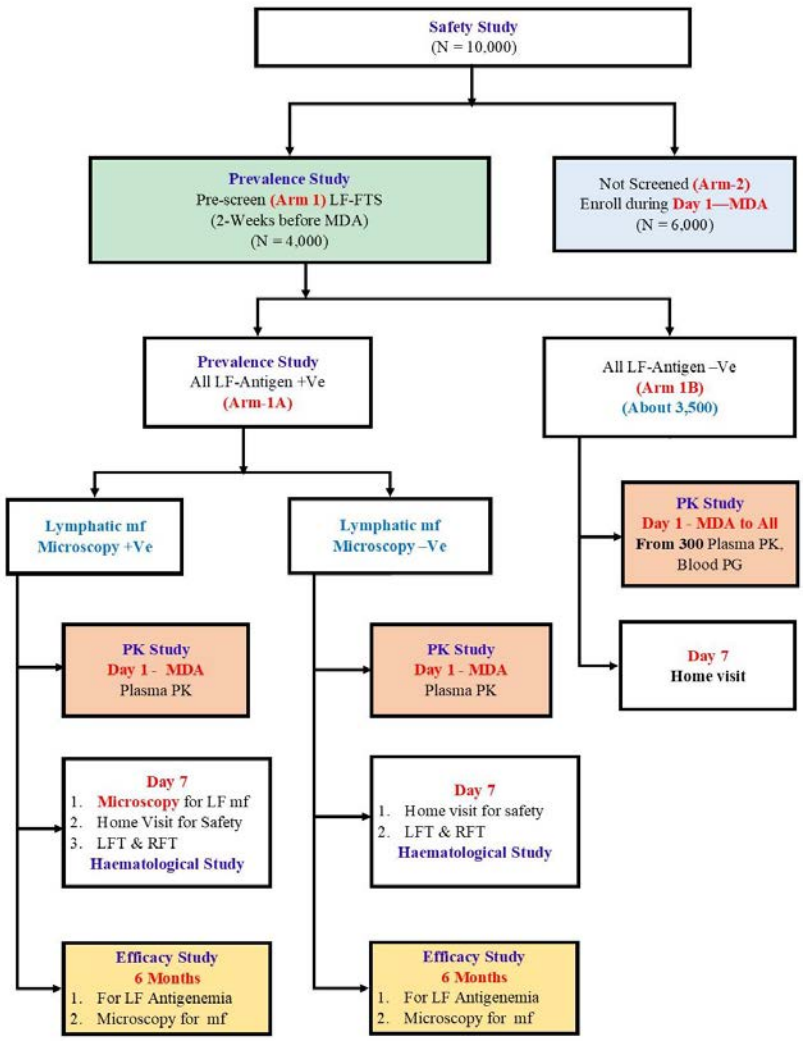


Figure 8: *Study flow chart*

The initial intention of the study was to first screen individuals in the community to determine those who were CFA positive and negative. This is because individuals with parasitaemia experience more AEs as compared to those without, due to an inflammatory response that occurs in those harbouring adult worms. The prevalence study therefore pre-screened 4,000 individuals to determine their CFA status. In this study, a cross-sectional study design was adopted. A purposive sampling technique was used to select 15 villages based on the past transmission trends, as assessed by CFA [39]. A convenient technique was further employed to recruit individuals with clinical conditions and laboratory results consistent with study objectives. The sampled villages were backed up with a review of epidemiological data from NTDCP. Study villages selected included Kichalikani, Kizingani, Kwale, Mongavyeru, Mwandusi, Tawalani, Manza, Kichakamiba, Subutuni, Zingibari, Moa, Ndumbani, Mayomboni, Bamba-Mwarongo (B'Mwarongo), and Maramba A.

The safety study then followed whereby 10,000 individuals were targeted for follow-up to document AEs occurring to them. In this study, a prospective, longitudinal, active CEM following MDA of IA was adopted. A cohort size of 10,000 individuals was targeted based on the assumption that a cohort of 3000 individuals gives a 95% probability of identifying a single AE with an incidence of 1:1000 [54, 55]. For a meaningful assessment, at least three events need to be identified and therefore this was the objective of aiming a larger sample size of 10,000 individuals [55, 133].

The second safety study utilized the analytical prospective longitudinal design to follow up individuals to determine the short–term effects of IA following MDA on the haematological parameters, liver and renal functions, and their relationship with safety. This study was nested in a bigger active surveillance study and data of 499 individuals was evaluated. Blood samples for haematological and biochemical testing were taken before and after uptake of IA (i.e., on days 0 and 7 respectively).

The PK study was also nested in an active surveillance safety study which aimed at investigating the PK disposition of IVM in 468 individuals. The method for detection of IVM was adopted and modified through mobile phase composition, column type, and extraction procedure to meet the optimal separation conditions. Plasma levels were quantified using the Duthaler *et. al.* LCMS/MS method [134]. The POPPK model was further developed by adapting a reference model from the literature. This was achieved by following the recommended best practices for using the PRIOR subroutine in NONMEM [135].

In the efficacy study, of the 4,115 individuals who were pre-screened in the prevalence study, 239 turned out to be CFA positive before MDA and were again tested for microscopic examination of mf seven days after MDA, which was again repeated six months later.

3.3 Data collection methods

3.3.1 Screening for CFA and mf

In the prevalence study (***Paper I***), all individuals were screened for CFA using the Alere™ Filariasis Test Strip (FTS) (Alere©, Waltham, United States). A finger prick was made using a sterile disposable lancet to collect blood samples from individuals. A special pipette with a 75 µL mark was used to apply approximately 75 µL of blood on the FTS and results were read in 10 min. FTS detects CFA released in the blood by adult worms (antigenemia).

For a positive confirmation of adult worms in blood one has to conduct a microscopy test to vividly see the worms after magnification. In this case, all antigenemia-positive individuals were again enrolled for night blood sampling with subsequent determination of mf. Blood sampling was done at a health facility or village office building. For those who did not show up after day blood sampling, they were followed up during the night through door-to-door visits. Finger prick blood samples were then taken from each at night between 21.00 and 01.00 h and poured onto 75 µL capillary tubes. Each blood sample was dispensed into a 1.8 mL cryo-tube containing 900 µL of 3% acetic acid, mixed thoroughly, and transferred into Sedgwick-Rafter counting chamber. The presence of mf was confirmed by counting their number in the chamber, using a compound light microscope set at 4×magnification. The microscopes were stationed at the NIMR laboratory in Tanga. Results were reported as the number of mf per 75 µL of blood.

3.3.2 Safety data collection and severity grading

In the safety study (***Paper II***) data was collected through pre- and post-MDA questionnaires. Pre-MDA questionnaires were used to collect data before MDA whereby consented individuals were asked to provide details on socio-demographics, clinical/medical history, any comorbidities, concomitant medications, and current clinical symptoms. These were recorded for comparison post-MDA. On the MDA Day, study participants received a standard dose of IVM 150–200 µg/kg and ALB 400 mg through CDDs who delivered the medications using the DOT approach. Study participants were then actively followed up for any treatment-related AEs on days 1, 2, and 7 following MDA. A cohort size of 10,000 individuals eligible to receive IA was determined based on the assumption that a cohort of 3,000 individuals gives a 95% probability of identifying a single AE with

an incidence of 1:1000 [54-56]. At least three events were needed to be identified, hence the rationale of obtaining a larger sample size of 10,000 individuals.

Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 [136] were used to grade the severity of observed AEs (1 to 5) as *mild, moderate, severe, potentially life-threatening,* and *death* as shown in **Table 2** below:

Table 2: Severity grading of adverse events after MDA

Grade	Severity
Grade 1	Mild - asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2	Moderate - minimal, local, or non-invasive intervention indicated, limiting age-appropriate instrumental Activities of Daily Living (ADL)
Grade 3	Severe or medically significant but not immediately life-threatening - hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
Grade 4	Life-threatening consequences - urgent intervention indicated
Grade 5	Death related to AE

3.3.3 Measurement of biological parameters

In ***Paper III***, haematological and biochemical parameters were determined at baseline before medicines were taken by study participants and on day 7 following drug administration. Complete blood count (CBC) was measured from whole blood collected in EDTA tubes. The tests were run using a Sysmex XS 1000i, a five-part differential cell counter (Kobe, Japan). White blood cells, WBC (10,000/µl), Red blood cells, RBC (million cells/mcL), Haemoglobin, Hb (g/dl), Platelets, PLT (1,000/µl), differential white blood cell count (mm3) and other blood-related parameters were determined.

Collected serum was used to measure renal and kidney function tests in a wet enzymatic detection system with a semi-automated Cobas C111 analyser (Roche Diagnostics, Switzerland). Parameters measured embraced alanine aminotransferase (ALT) (IU/l), creatinine (µmol/l), alkaline phosphatase (ALP) (IU/L), aspartate aminotransferase (AST) (IU/L), direct bilirubin (BilD) (µmol/l) and total bilirubin (BilT) (mol/l).

Both haematological and biochemical parameters were classified as normal or abnormal based on the reference range. Haematological parameters which included WBC, RBC, Hb, HCT, MCV, MCH, MCHC, PLT, PDW, MPV, PLCR, PCT, lymphocytes, monocytes, neutrophils, and eosinophils were classified as

abnormal when their values were below the lower limit of the reference range. Biochemical parameters which included serum creatinine, ALT, AST, BilD, and BilT were classified as abnormal when their values were above the upper limit of the reference range.

3.3.4 Pharmacokinetics analysis including POPPK modelling

In the PK study (***Paper IV***), study participants were given a standard dose of IVM based on height (roughly corresponding to 150–200 µg/kg). Individuals weighing > 15 Kg received 3-, 6-, 9-, or 12 mg if their height were > 90 cm and ≤ 119 cm, > 119 cm and ≤ 139 cm, > 139 cm and ≤ 159 cm, >159 cm, respectively. All individuals received ALB 400 mg. Individuals < 15 Kg did not participate in the MDA programme. Two mL of venous blood were collected from the antecubital arm veins of participating individuals at 0-, 2-, 4-, and 6 hours following drug administration. Plasma levels of IVM were quantified using the Duthaler *et. al.* LCMS/MS method with modifications [134].

The POPPK analysis was performed using NONMEM due to limited time points obtained in the PK study. A two-compartment model with first-order absorption and disposition kinetics was used. The AUC0-∞ and terminal half-life were successfully predicted using the simulated model. Demographics and clinical and pharmacogenetic (PG) characteristics were included in the model to assess their influence on the PK of IVM.

3.3.5 Evaluation of IVM height-based dosing

During MDA in Tanzania, IVM is always administered based on height. This is due to logistical challenges and the feasibility of supplying weighing scales in rural communities. Considering the variability in dose administered, and as part of ***Paper IV***, we assessed if height-based dosing of IVM results in doses >150 µg/kg in different weight bands (i.e., 15-30, 31-40, 41-50, 51-60, 61-70, 71-80, and 80-90 Kg). Doses that individuals received were divided by weight. Individuals were grouped into weight-bands and the proportion of individuals receiving < 150 µg/kg in each weight-band was calculated. We also assessed if the 200 µg/kg dosing followed by rounding to the nearest number of whole tablets achieved doses > 150 µg/Kg in different weight bands. Individuals' weights were multiplied by 200 µg/Kg and the resulting dose was rounded to the nearest number of whole tablets. The final dose was divided by body weight and the proportion of individuals receiving <150 µg/kg in each weight category was calculated. Finally, model-based simulation was used to compare IVM exposure (AUC0-∞) across weight bands after the current weight- or height-based dosing.

3.3.6 Efficacy Data Collection

In the efficacy study (***Paper V***), 239 individuals who were CFA positive in the prevalence study (***Paper I***) were enrolled. Assessment of antigenemia and detection of mf were as in ***Paper I***. This exercise was, nonetheless, executed before MDA and repeated seven days and six months after MDA. Furthermore, the Cmax and AUC were assessed for between-subject variability (coefficient of variation - CV%). The association of variability in IVM PK parameters with clearance of mf and CFA after MDA was analysed as well.

3.4 Data Management and Statistical Analysis

Data was collected electronically using tablets and submitted daily to the central server at the NIMR laboratory in Tanga. The open-source data kit (ODK, https://opendatakit.org/) software was used to create the database and data collection applications. The data manager reviewed the collected data daily for precision and consistency and queries generated were sent back to the head of each study team for resolution. Data cleaning and validation were done continuously, and periodic reports were generated. Final cleaning was done after the surveillance, and analysis was performed according to the data analysis plan.

In ***Paper I***, data were analysed using STATA version 13 and R statistical software (version 4.1.2; www.r-project.org). Descriptive statistics was used to calculate the proportion of study participants who were CFA and mf positive, and the mean mf parasite intensity. Continuous variables with normal distribution were compared using a t-test or ANOVA test. Skewed continuous variables were presented as median with interquartile ranges (IQR), and were compared using sign rank tests, while categorical data were compared using the Chi-square ($\chi 2$) test. $\chi 2$ test was also used to determine whether age was associated with CFA and scrotal enlargement. Binary logistic regression analyses were performed to generate odds ratios (OR) including 95% confidence intervals (95% CI) to assess potential relationships between age, sex, missing MDA, living in hotspots (exposures), and CFA positivity (outcome).

In ***Paper II,*** data were collected and managed as in ***Paper I,*** and categorical variables were summarized as proportions, while continuous variables were summarized as mean with standard deviation (SD) or median and IQR. Categorical variables were compared using the $\chi 2$ test. Both univariate and multivariate logistic regression models were used to determine factors associated with any AEs. Significance levels in ***Paper I*** and ***II*** studies were all set at 0.05 and the confidence interval at 95%. All p-values were two-sided and a p-value of less than or equal to 0.05 was considered to indicate statistical significance.

In ***Paper III,*** the electronically collected data were extracted into an Excel format and then imported to IBM Corp. 2019, IBM SPSS Statistics for Windows, Version 26.0 for analysis. Missing pattern analysis was conducted to assess the extent of missing values in each variable. Little Missing Completely at Random (Little's MCAR) test indicated that the missing dataset was not completely at random ($\chi 2$ = 2628.5, df = 2028, $p < 0.001$), and therefore imputation was adopted. Multiple imputation was conducted in R (version 4.3.2); using mice package (version 3.16.0). Descriptive statistics were used to describe study participants' socio-demographic and clinical characteristics. Chi-square and Fisher's exact tests were used to determine the association between CFA status and sociodemographic characteristics at baseline. Mann-Whitney U test was used to compare baseline haematological and biochemical parameters between CFA positive and negative groups. Comparison of laboratory results in unstratified, CFA positive and negative individuals before and after MDA was carried out using Wilcoxon signed rank test. McNemar test was used to compare the proportion of individuals with altered haematological and biochemical parameters before and after MDA. Binary logistic regression was used to determine the predictors of abnormal Hb concentration. Variables with p-value < 0.2 in univariable analysis were included in the multivariable model. A p-value <0.05 was considered statistically significant. A heat map was drawn to show the relationship between AEs and changes in laboratory parameters post-MDA.

In ***Paper IV***, the POPPK modelling was done using NONMEM (version 7.5.0, Icon Development Solutions, http://www.iconplc.com, Ellicott City, MD, USA) with PsN (version 5.2.6; https://uupharmacometrics.github.io/PsN/) and Pirana (version 2.9.9; https://www.certara.com/software/pirana-modeling-workbench/). Data preparation and NONMEM results post-processing and visualization was conducted using the R statistical software (version 4.1.2; www.r-project.org). The maximum likelihood estimation method (FOCE-I in NONMEM) was used for parameter estimation. Models were evaluated using the likelihood ratio test, goodness of fit (GOF) plots, parameter uncertainty, and visual predictive checks (VPC). The difference in OFV >3.84 units (equivalent to p-value ≤0.05 for $\chi 2$ distribution) between nested models was considered a statistically significant improvement in model fit. Uncertainties of model parameters were measured by RSE or bootstrap CI for the final model. Inter-individual variability (IIV) of model parameters, as measured by coefficient of variation (CV%), was also calculated. Potential covariates were identified by visual inspection of covariates versus η plots. Only biologically plausible covariates were added to the model by manual stepwise forward inclusion only. Covariates were retained in the model if their addition led to a≥3.84 decrease in OFV (i.e., p-value ≤ 0.05). Mixture modeling was

further used to find any unknown sub-populations with different typical parameter estimates compared to the main population. The final model was qualified by GOF plots, VPC, and non-parametric CI obtained by fitting the final model to 200 bootstrapped samples of the current dataset (bootstrap confidence intervals).

In ***Paper V***, descriptive statistics summarized sociodemographic and clinical characteristics. To assess the impact of MDA, the McNemar test was employed to compare the proportions of microfilariae positives before and after the intervention. Factors associated with CFA status at six months were analysed using either the Chi-square or Fisher's exact tests. Univariable and multivariable logistic regression analyses were done to control for potential confounders. Factors with a p-value of <0.2 in the univariable analysis or with clinical relevance and previously reported associations were included in the multivariable model. All p-values in statistical tests were two-sided, with a significance level set at <0.05. Data visualization and analysis were performed using GraphPad Prism version 10 (California, USA).

3.5 Ethical Considerations

Before the commencement of all studies, ethical clearance was granted by the Medical Research Coordinating Committee (MRCC) of the National Institute for Medical Research, Tanzania (Certificate No. NIMR/HQ/R.8a/Vol. IX/2890 dated 17th September 2018). Initial meetings with district and village authorities were conducted explaining the aim, objectives, and mechanisms for recruiting study participants living in the study area. This was followed by community sensitization meetings, which were conducted in each village to inform the community about the purpose, methodology, and significance of the proposed research, and to obtain community consent. Questions were asked, and explanations were provided, by researchers during these meetings. Informed consent was then obtained in writing from all individual participants and/or parents/guardians in the case of children. Confidentiality of individuals' information was maintained during and after the study.

4 Results

This PhD project was divided into two main components – one which intended to actively follow up on safety and the other which aimed at determining the efficacy of MDA drugs. Screening of individuals was done before MDA to determine the prevalence of LF and estimate the number of individuals who were antigenemic and microfilaremic to be included in the safety and efficacy analysis. In the screening exercise, blood samples were also drawn for haematological and biochemical assessment as well as PK analysis.

4.1 Prevalence and Correlates of LF Infection and its Morbidity Following MDA

In the prevalence study (***Paper I***) a total of 4115 individuals from 15 villages in Mkinga district were screened for CFA, of which 2045 were males (49.7%) and 1447 (35.2%) children aged 5–14 years. A variation in the age of the study participants was noted in villages with some having the oldest population (median age 32 years), and others the youngest (14 years). The median age of participants was 22.7 years (IQR = 12.5–44.5 years), being significantly higher among females (median=25.6, IQR=12.8–45.0) than males (median=20.0 years, IQR=12.2–43.9), p=0.008.

4.1.1 Prevalence and Correlates of CFA

The overall prevalence of CFA positivity was 5.8% (239/4115), 95%CI: 5.1–6.6), with males having significantly higher rates (8.3%) compared to females (3.3%), p<0.001. A positive trend of increase in the prevalence of CFA positivity with increasing age was also noted (χ^2= 53.83, p<0.001), with a maximum of 10.2% being among the age group of 65+ years. The pattern of LF infection by age was also similar between females and males. However, after adjusting for the effect of age, the risk (odds ratio) for being positive in males was 2.9-fold higher (95%CI: 2.14–3.85, p<0.001) when compared to females. The prevalence of antigenemia varied significantly between villages ranging from 1.2% to 13.5%. After regression analysis, old age, male sex, missing last MDA, never taking IVM, and living in Kwale, Maramba, or Moa wards were significant correlates of CFA positivity. Maramba A is the hotspot for LF in the Mkinga district.

4.1.2 Prevalence and correlates of microfilaremia

Out of 239 individuals who tested positive for CFA, 28 (11.7%) were lost to follow-up for the night blood specimen collection for the detection of mf using microscopy. Eleven individuals out of 211 were mf-positive, giving a prevalence of 5.2% among CFA-positive individuals. Microfilaraemia was detected in 4 out of 15 villages. The youngest mf positive child was 11 years old.

4.1.3 Coverage of MDA from the previous round

Epidemiological coverage during MDA measures the proportion of individuals who administered the medications amongst the total population in the implementation unit. WHO recommends the minimum epidemiological coverage for MDA to be considered effective for reducing LF transmission to be at 65% or greater. Coverage of MDA during the last round of distribution (i.e., 2017) was explored in 3,932 individuals from the age of 6 years, of which 2379 responded to have ingested the medications (60.5%). The proportion of individuals who did not take MDA drugs (i.e., 1553 (39.5%)) was slightly higher in males (40.9%) than in females (37.9%), p=0.06.

It was further observed that out of 3,846 individuals, 1339 (34.8%) had never used IVM before with a slightly higher frequency seen in males (36.2%) than in females (33.4%). The prevalence of CFA-positive individuals who did not take MDA drugs in the previous year (8.25%, 134/1625) was significantly higher than those who took (4.18%, 100/2392) (χ^2 = 29.15, p< 0.0001, odds ratio =2.056; 95% CI of OR = 1.5771 to 2.6905). The proportion of individuals who did not take MDA during the last round or never used IVM before was lower in children aged 11–15 years (18.8%) than in adults. There were significant differences in the proportion of individuals who missed MDA in the previous year amongst the various age groups, the highest being in the 25–34 years age group (52.3%) and 65+ (52.3%) p< 0.0001. The trend of use of MDA in the previous year by age group coincided with the CFA positivity, except for the youngest age group. The same pattern of high CFA positivity was also observed in villages with a history of low MDA compliance during the last round.

4.1.4 Clinical manifestations of LF

Classical clinical manifestations of LF which are usually monitored by the NTDCP include scrotal swelling, hydrocele, swelling of arms or legs, lymphoedema, and lymphadenopathy. Scrotal swelling is an enlargement of the scrotal sac or scrotum which houses the testicles. Scrotal enlargement was assessed for 1,988 males, of which 128 (6.4%) had an enlarged scrotum. The rate of scrotal enlargement was increasing with age (χ^2_{trend}=126.3, p<0.001). Scrotum enlargement was significantly higher among males with positive CFA (14.8%), compared to those with a negative test (7.9%), p=0.006. Hydrocele which is a type of swelling in the scrotum that occurs when fluid collects in the thin sheath surrounding the testicles, was detected in 73/1992 (3.7%) males, which was 57.5% (73/127) of those with an enlarged scrotum. Nonetheless, hydrocele prevalence was not significantly different between those with a positive CFA test (4.8%) against those with a negative test (3.6%), p= 0.42.

Swelling of arms or legs was observed at a lower frequency of 55/4074 (1.35%), and these were mainly in individuals with negative CFA tests (53 cases), although there was a similar pattern with other types of swellings. A variation in the prevalence of swelling within the village of residence was interestingly noticed, albeit the pattern did not mimic the CFA positivity trend, as villages with the highest CFA prevalence were observed to have a lower prevalence of swellings.

Lymphoedema and lymphadenopathy were also observed at a low frequency whereby the prevalence of lymphoedema was 1.2% (50/4059), while lymphadenopathy was 0.32% (13/4065).

4.2 Safety and Tolerability of MDA Drugs Deployed in LF Endemic Communities

In this safety and tolerability study (***Paper II***) a total of 9640 individuals from 24 villages were followed up. The study adopted the CEM method for active surveillance of individuals in their households. Before MDA, socio-demographics, clinical conditions, co-medications, use of traditional and herbal medicines, etc. were recorded. On the day of MDA, individuals were given IA which was administered by CDDs. Of the enrolled individuals, 9288 (96.3%) completed the seven-day safety follow-up. Amongst these 9,288 individuals from whom post-MDA safety data were collected, 4,816 (51.9%) were females.

4.2.1 Incidence of Adverse Events Following MDA

CEM allows for the measurement of incidence rates and minimizes losses to follow-up. The follow-up rate in this study was 96.3%. A total of 352 (3.7%) individuals were lost to follow-up and were not included in the final analysis. Before MDA 1,312 individuals reported their clinical symptoms which were recorded as pre-MDA events for a comparison post-MDA. Any reported post-MDA event was cross-checked and verified to differentiate MDA-associated AEs from pre-existing clinical symptoms.

Out of the 9288 from whom follow-up data were recorded, 442 individuals reported at least one post-MDA AE. The overall incidence of experiencing at least one post-MDA AE was 4.8% (95% CI = 4.3–5.2%). The proportion of individuals who reported one, two, and three or more events was 2.8% (n = 260), 1.3% (n = 122), and 0.6% (n = 60), respectively.

Of the 1,312 individuals who reported any clinical symptom before taking MDA, 111 (8.5%, 95% CI = 4.3–5.2%) reported at least one new symptom after taking MDA (post-MDA AE). Among 7,976 individuals who did not report pre-MDA symptoms, 331 (4.1%, 95% CI = 3.7–4.6%) experienced at least one type of post-MDA AE. Participants with underlying pre-MDA clinical symptoms had a significantly higher risk of experiencing MDA-associated AE ($p < 0.001$, odds ratio = 2.13: 95% CI = 1.71–

2.67). The incidence of AEs decreased by day during the follow-up period compared to day one ($p < 0.001$).

4.2.2 Incidence stratified by types of MDA-associated AEs

Generally, more AEs were observed in day one and decreased progressively until day seven. The most common AEs with relatively higher incidence rates were headache (1.23%), drowsiness (1.15%), fever (1.12%), dizziness (1.06%), and abdominal pain (0.88%), while confusion, vomiting, and difficulty in breathing had the lowest rates throughout the follow-up period.

4.2.3 Severity grading of MDA-associated AEs

Most of the reported post-MDA AEs were mild (83.8%) and moderate (15.9%), with few that were severe (0.3%). Only two individuals reported severe drowsiness (0.9%) and dizziness (1%).

4.2.4 Correlates and predictors of AEs following MDA

The incidence rates of AEs were significantly higher among females and those who had chronic illnesses and chronic manifestations of LF ($p < 0.001$). The incidence of AEs was not statistically different between the different age groups, those who used bed nets, and those who participated in the previous MDA round. Likewise, AEs were not significantly associated with the use of traditional medicines nor the number of IVM tablets administered.

4.3 Surveillance of Haematological and Biochemical Changes Following MDA

This extended safety study (***Paper III***) extracted data from a cohort of 499 individuals who were screened in the prevalence study (***Paper I***). In this study group, blood samples were drawn from individuals for laboratory analysis of haematological and biochemical parameters to investigate their changing patterns. Out of 499 individuals followed up, 174 (34.9%) were CFA positive. The distribution of age was significantly different between CFA positive and negative groups ($p<0.001$). The majority of individuals in both groups were aged between 19 and 59 years. The median age was significantly higher in the CFA positive as compared to the CFA negative group. Overall, the study enrolled more males than females ($p=0.017$). The median height and weight (IQR) were significantly higher in CFA-positive as compared to CFA-negative individuals ($p<0.001$). The proportion of individuals in BMI categories differed significantly between CFA groups. Individuals with normal BMI were more in the CFA positive as compared to the CFA negative group ($p<0.001$). Individuals with experience of taking IA in previous MDA rounds were significantly more in the CFA negative as compared to the CFA positive group ($p=0.042$).

4.3.1 Comparison of baseline laboratory parameters by CFA status

At baseline before MDA, median values for HCT, eosinophils, serum creatinine, bilirubin direct, and bilirubin total were significantly higher in the CFA-positive group than the CFA-negative group. The median PCT and ALP levels were significantly lower in CFA-positive individuals than in CFA-negative. Other parameters were not statistically different between the two groups.

4.3.2 Change in haematological and biochemical parameters after MDA

Among individuals who were CFA positive, there was a statistically significant increase in the median values of MCH, MCHC, MPV, PLCR, monocytes, AST, and creatinine after MDA ($p<0.05$). On the flip side, there was a statistically significant decrease in median values of RBC, Hb, HCT, BilD, and BilT following MDA ($p<0.05$). In the CFA-negative individuals, there was a statistically significant increase in the median MCH, MCHC, MPV, PLCR, Mono, EOs, BilD, and BilT values after MDA. A substantial decline in median RBC, Hb, HCT, and ALT levels following MDA was further observed ($p<0.05$)

4.3.3 Proportion of abnormalities in haematological and biochemical parameters after MDA

Among individuals who had normal haematological and biochemical parameters at baseline, 13.3%, 30.0%, 43.8%, 43.2%, 20.1%, 25.0%, 8.2%, 25.2%, 4.7%, and 07.7%% had a significant decrease in values of RBC, Hb, HCT, MCH, MCHC, monocytes, eosinophils, creatinine, and ALT respectively. All other parameters had no significant changes from the normal reference range. The univariable and multivariable logistic regression did not reveal any significant predictor of low Hb concentration following MDA.

4.3.4 Association of haematological and biochemical abnormalities with adverse events following MDA

Adverse events following MDA were actively monitored in 403 study participants. Thirty-two individuals (7.9%, 95% CI = 5.3 - 10.6) reported at least one type of MDA-associated AE during a 7-day follow-up. Dizziness, drowsiness, and headache were the most reported AEs, occurring at an incidence rate of 2.5% each. There were no significant differences in the incidence of AEs between CFA-positive (7.9%, 11/139) and CFA-negative individuals (8.0%, 21/264). Correlation of AEs with change in haematological and biochemical parameters after MDA was tested. AEs amongst individuals (n=19) with abnormal haematological and biochemical parameters following MDA were observed and reported. Drowsiness was the most observed AE among individuals with abnormal parameters, followed by fever, dizziness, and nausea.

4.4 Population Pharmacokinetics of IVM Using a Modelling Analysis Approach

In this PK study (***Paper IV***) we aimed to determine the PK profile of IVM only. The initial intention was to measure all PK parameters including Cmax, Tmax, and AUC. However, at the time of analysis, we could not estimate the $AUC_{0-\infty}$ and elimination half-life which are critical PK parameters for determining drug exposure as a measure of bioavailability. We therefore had to adopt a POPPK model using the 4 sampling time points to obtain the intended PK parameters and explore the predictors of IVM disposition.

4.4.1 Population Pharmacokinetics Modelling

The dataset for POPPK modelling consisted of 468 individuals (males n= 279; 59.9%) with a total of 1404 PK samples collected at 0-, 2-, 4-, and 6-hours after a single dose of IVM. Over half of the enrolled individuals (n=248; 53%) received a dose of 12 mg. P values were calculated for each variable to test for any significant differences in baseline characteristics among individuals who received different doses. Enlarged scrotum, lymphadenopathy, lymphoedema, hydrocele, WBCs, and monocytes were variables that did not show statistical differences among individuals in different doses ($p>0.05$).

Parameters of the model were apparent clearance (CL/F), apparent central volume (Vc/F), apparent inter-compartment clearance (Q/F), apparent peripheral volume (Vp/F), absorption rate constant (Ka), mean transit time (MTT) and number of transit compartments (NN). Allometric scaling was applied to clearance (CL/F) and volume (Vc/F) with fixed allometric exponents. Refitting the model with our data led to a huge decrease in OFV (ΔOFV=-33664). Since we also aimed at testing covariates for bioavailability, we estimated the IIV for bioavailability (IIV-F1) while F1 was fixed to 1. This led to a further decrease in OFV (ΔOFV=-13), and therefore the model with IIV-F1 became our base model.

Covariates versus η plots indicated that only the 3mg dose was a potential covariate on bioavailability (F1). The η distribution plots indicated the right-skewed distribution of IIV-MTT. The addition of the 3 mg dose as a covariate on F1 led to improved model fit (ΔOFV=-10.0), while mixture modelling of the MTT parameter identified two sub-populations and resulted in statistically significant improvement in model fit (ΔOFV=-17).

The RSEs were consistent with the narrow bootstrap CI and indicated that the parameters were estimated with good precision. Dose was the only identified covariate of F1 as the 3mg doses had about 48% higher bioavailability compared to the other higher doses. Based on the GOF plots, the developed model provided an adequate description of the current data. Similarly, the comparison of observed

and model-estimated individual secondary PK parameters indicated that model estimates were in good agreement with the observed data. The model estimated AUC0-∞ indicated that individuals who received 3 mg doses had comparable exposure to the rest of the study participants who received 6-, 9-, and 12-mg doses.

4.4.2 IVM dosing by height and weight

Based on the current height-based dosing, 6.3% of individuals weighing <30Kg were under-dosed (i.e., received < 150 µg/Kg) while about 23%, 30%, and 100% of individuals in the weight range of 61-70 kg, 71-80 Kg, and > 80 Kg, respectively, were under-dosed. With the 200 µg/Kg dose, rounded to the nearest number of whole IVM tablets (i.e., 3 mg per tablet), only individuals weighing <30Kg would be under-dosed; about 17.5% of these would receive <150µg/Kg due to rounding to the nearest number of whole tablets. These findings were supported by results from model-based simulations which showed that contrary to weight-based dosing, height-based dosing results in relatively lower AUC0-∞ for individuals weighing >70Kg compared to those weighing<70Kg.

4.5 Efficacy of MDA Drugs

In the efficacy study (***Paper V***), a cohort of 239 CFA-positive individuals out of 4115 screened, was enrolled. Out of the 11 individuals who were mf positive at baseline, 10 were available for a follow-up test on day 7. Amongst these 10 individuals, 9 (90%) tested negative for mf on day 7 post-MDA. One individual who remained mf positive on day 7 showed a decrease in parasite counts from 27 to 13 microfilariae/mL. The McNemar test indicated a significant change in mf status before and after MDA.

At six-month follow-up, a total of 168 individuals were available for night blood sampling for mf microscopy. Amongst those who were mf-free on day 7, all remained mf-negative at six months post-MDA - including the nine individuals who had cleared mf. Alternatively, the two individuals who tested positive for mf on day 7 remained mf-positive after six months.

Individuals who cleared mf on day 7 had minimal inter-individual variability in drug exposure as measured by Cmax and AUC. The Cmax of IVM ranged between 47.9 and 77.4 ng/mL (CV=19.2%) while AUC0-∞ and AUC0–6h were 802.3 to 1402.8 ng.h/mL (CV=20.7%) and 149.77 to 276.01 ng.h/mL (CV=19.9%), respectively. The individual who did not clear mf on day 7 had a comparable exposure.

Factors associated with CFA status after 6 months following MDA were determined using Chi-square or Fishers exact tests. Area of residence (ward) was the only significant factor associated with CFA status, whereby residents of

Maramba ward significantly cleared CFA levels (37.5%) compared to other wards (p=0.024). All other factors were not significantly associated with CFA status.

There were no significant differences in the geometric mean of IVM Cmax (p = 0.19) and AUC (p= 0.41) of between CFA-positive and negative individuals at six months of receiving IA. The univariable and multivariable analyses revealed that none of the factors tested were significantly associated with CFA clearance at six months following MDA (p>0.05). Factors included in the multivariable model were based on clinical relevance and previously reported associations.

5 Discussion

Overall, a total of 9,640 individuals were enrolled and followed up for safety evaluation in this PhD project, of which 4,115 were pre-screened for prior CFA status, 499 tested for haematological and biochemical changing patterns, and 468 were assessed for PK variability at pre-dose-and-at 2-, 4-, and 6-hours post-dose. The efficacy of IA was further assessed in 239 individuals by measuring the clearance of microfilaraemia and antigenemia at day 7, and six-months post MDA.

5.1 Prevalence and Correlates of LF Infection and its Morbidity (Paper I)

In the prevalence study (***Paper I***), 4115 individuals from 15 villages in the Mkinga district were screened for CFA status. Mkinga district is one of the highly LF endemic areas in north-eastern Tanzania. The overall intention of this study was to pre-screen individuals before subjecting them to the safety, efficacy, and PK studies. We aimed to identify a cohort of individuals with the disease and those without by measuring antigenemia levels and determining predictors of safety and PK in the follow-up sub-studies.

5.1.1 Prevalence and correlates of infection

In this study, the status of LF infection was investigated and the overall prevalence of antigenemia positivity was 5.8%. The prevalence of microfilaraemia amongst those who tested positive for antigenemia was 5.2%. The prevalence of antigenemia was significantly higher in males (8.3%) than females (3.3%), and positively correlated with increasing age. The observed high prevalence of antigenemia in males as compared to females was in tandem with the results from other studies [22, 24, 36, 137]. Most men in the Mkinga district spend much of their time outdoors until late hours which is the time of mosquito bites. Fishing is also one of the main economic activities for them to earn their living. It is due to these socioeconomic activities that predispose them to late-night mosquito bites and therefore transmission of the disease.

The presence of CFA in blood indicates exposure of an individual to worms and such antigens are expressed after disease transmission. The existence of these antigens in blood also signifies that an individual has acquired infection as filarial worms take time to mature as they develop into adult worms. However, it is not clear for how long antigenemia subsides after the exposure of individuals. Adult worms can live for up to 8 years producing millions of mf that continue to circulate in the blood causing clinical manifestations [14, 30]. The increasing trend of CFA positivity with age signified the role of adult parasites as they mature in infected individuals. The combination of IA used during MDA is effective against the mf (immature larvae stage) but not the matured adult worms. The timing sustained,

and uninterrupted MDA programme is, therefore, crucial to ensure that mf is killed to stop the transmission of the disease.

To compare the prevalence rates in the same district, we evaluated data from the NTD programme. The prevalence of LF was 62.0%, 3.70%, and 6.0% in 2002, 2014 and 2017 respectively. According to this data, there was a sharp decline in the prevalence of LF between 2002 and 2014. The CFA-positivity of 6% which was reported by NTCDP in 2017 is comparable to the overall prevalence of 5.8% observed in our study – a year later.

Since the launching of GPELF in 2000, more than 18 years had elapsed at the time of this prevalence study in 2018. Epidemiological surveys conducted by WHO in different settings have reported that LF is still prevalent in many countries. As of 2018, 51 million people were infected – a 74% decline since the beginning of GPELF [14]. Due to this current status quo, the WHO has recently decided to shift the goal-post and now the GEPLF targets have been shifted to 2030. A strategic blueprint to guide actions to control, eliminate, or eradicate 20 diseases and NTDs including LF over the next decade was published by WHO in 2020 [138]. The blueprint highlights a roadmap to end NTDs by 2030 as part of sustaining development goals. According to this blueprint, drivers of success include continued donation of drugs by pharmaceutical companies, multisectoral collaboration, and continuous innovation in new treatments and/or diagnostics. Additionally, actions that have been suggested by WHO to meet the new targets include securing sufficient resources to enable programme implementation, mainstreaming NTD interventions into national health systems as an integrated part of primary health care, encouraging closer collaboration across all relevant sectors, and pursuing research and development on innovative tools and methods to combat NTDs.

5.1.2 MDA coverage in the previous year

Adequate coverage of the MDA round in an implementation unit is critical for the successful elimination of LF. The WHO recommends an effective coverage of >65% of the targeted population [30]. In our study coverage of MDA in the previous year was measured at 60%. This means about 40% did not take MDA drugs in the previous year (i.e., 2017). The proportion of individuals who did not take MDA drugs was observed to be higher in males than females. Low MDA coverage reduces the impact of MDA on transmission and delays elimination. Missing MDA in the previous year was significantly associated with a high risk of CFA-positivity. Henceforth, low MDA coverage could be one of the contributing factors towards the slow elimination of LF in Tanzania. The proportion of individuals who missed MDA in the previous year was also high in individuals aged

15–34 years and beyond 65 years, indicating that non-compliance to MDA correlates with the observed high infection rates in these age groups.

Mkinga district being one of the highly endemic areas for LF, has experienced persistent infection rates since MDA began in 2002. Several rounds of MDA deploying IA had been staged in this district but to date, the prevalence of infection has not declined beyond the levels where recrudescence is unlikely to occur. The probable reason for this persistence was inherent low coverage and poor perception, knowledge, and attitude toward MDA. Cabral *et al.* assessed the knowledge, attitudes, and perceptions regarding LF in a study on systematic non-compliance with MDA in north-eastern Brazil [139]. In this study not receiving the drug and fear of side effects were observed as the most important causes of systematic non-compliance to MDA. People without knowledge of the disease and existing programmes to eliminate LF were also significant predictors of low coverage in a study done in Haiti [140]. Other similar studies have also demonstrated a lack of awareness to be associated with non–compliance to MDA [141-144]. Apart from awareness and knowledge, Krentel *et. al.* in their review of literature, also cited other major factors contributing to non-compliance to MDA including MDA setting, confidence in MDA, fear of AEs vs benefits, prior experience with MDA, recipient's situation and drug distributor [145].

In our study, males had a high tendency of missing MDA, due to their constant absence from their homes during MDA rounds. When CDDs distribute drugs, males are usually not available in their homes and most of them refuse to take such drugs because of misconceptions about their AEs as observed in the cited studies. There is a myth in many districts that once you use these medicines, you might end up impotent and fail to exercise sexual intercourse. This adds up to the low coverage of MDA and therefore continued transmission. It was therefore hypothesized that most men missed the previous round due to poor perception of MDA medicines that they can cause infertility and affect their reproductive capabilities.

MDA is needed to reduce infection in the community to levels below a threshold at which mosquitoes are unable to continue transmitting parasites and new infections are prevented. Pre-Transmission Assessment Survey (pre-TAS) is recommended to be conducted after five effective MDA rounds when the prevalence of infection is less than 1% for mf and 2% for CFA [30]. This should be followed by the Transmission Assessment Survey (TAS). In all these cases, the coverage should be >65% of an implementation unit. TAS measures whether evaluation units have lowered the prevalence to a level where recrudescence is unlikely to occur, and transmission is considered not possible even without MDA [30]. Repeated MDA rounds with adequate coverage are therefore crucial for LF

elimination. Non-compliance with MDA represents a serious programmatic hiccup for the LF programme because systematically non-compliant individuals serve as a reservoir for the parasite and thus the recrudescence of infection. The success of the LF control programme depends in large on its ability to achieve and sustain high levels of compliance to MDA.

5.1.3 Morbidity and disease manifestations

The WHO has classified LF as the second most common cause of long-term disability after mental illness [146]. Apart from interrupting transmission through MDA, preventing suffering and disability in those who already have chronic LF manifestations is part of GPELF strategies underpinned in the WHO 2030 target [138]. NTD programme monitors leg or arm swelling (lymphoedema) and scrotal swelling (hydrocele) during morbidity control as the full-blown disease is irreversible. Once such conditions occur to affected individuals what needs to be done is to manage lymphoedema and arrange for surgical interventions in case of hydrocele.

In our study, swelling of arms or legs was observed at a lower frequency of 1.35% (55/4074), and these were mainly in CFA-negative individuals. Hydrocele was at 3.7% (73/1992) of males, which was 57.5% (73/127) of those with an enlarged scrotum. Scrotal enlargement was measured at 6.4%, and the rate of enlargement was increasing with age. The same was significantly higher among males with positive CFA (14.8%), compared to those with a negative test. An important association between filarial infection and inadequate surgical and clinical management of hydrocele was identified as a risk factor for lymph scrotum in a case series study done by Aguiar-Santos [147].

There were reduced hydrocele and lymphoedema rates in the young age group (11–24 years) compared to the older group (≥25–65 years). This signified that the reduction of new infections resulted in a lowered progression of infection to chronic pathologies, compared to the findings of previous studies done in the same locality [36, 148]. Small scrotal swellings in the age group of 5–11 years were observed as a sign of sub-clinical hydrocele. The frequencies of hydrocele observed in this study were similar to those reported in other studies [8, 38]. Other studies have also demonstrated that filarial hydrocele is triggered by the death of an adult worm, which produces an inflammatory nodule that occludes the lymphatic vessel [149-151].

Since lymphoedema and hydrocele are the most frequent sequelae observed in endemic communities, the NTD programme should continue to monitor and consider offering services to those suffering from the clinical manifestations taking into account their social-economic status. Furthermore, much as our study focused on one single district, the programme should also contemplate

conducting more population-based surveys in other endemic areas of the country to provide more robust and reliable estimates of the burden of filarial morbidity.

5.2 Safety and Tolerability of IA Following MDA (Paper II)

No drug is absolutely safe after being authorized for marketing by National Medicines Regulatory Authorities (NMRAs). Continuous monitoring is mandatory for the entire life circle of the drug in circulation. NMRAs need to set up robust and effective PV systems that can detect, understand, assess, and prevent AEs and consequently ADRs. PV systems are weak in many countries particularly those with limited resources. MDA of IA in many settings has also been conducted without effective monitoring of AEs to be certain of the safety of such drugs. The safety study (***Paper II***) was therefore conducted to actively identify the incidence, timing, type, severity, and associated risk factors of AEs following MDA in the Mkinga district. Clinical symptoms before drug intake were recorded and cross-checked with post-MDA reported events to differentiate treatment-associated AEs from any pre-existing clinical symptoms. The overall cumulative incidence of experiencing at least one type of MDA-associated AE was 4.8%; this being significantly higher among those who had pre-existing clinical conditions (8.5%) than those without (4.1%). Most of the observed AEs were mild (83.8%) and moderate (15.9%), with few severe (0.3%). Most AEs that occurred during the first two days of MDA were transient and resolved progressively to day seven. Headache, drowsiness, fever, dizziness, and stomach pain were the most common AEs.

Observational studies and clinical trials conducted elsewhere had also reported common AEs associated with IVM such as headache, pruritus, muscle pain, cough, dyspnoea, nausea, vomiting, diarrhoea, blurred vision, postural hypotension, confusion, skin reactions and oedematous swellings [66, 152, 153]. AEs were also reported by 197 (1.2%) of individuals in a study that compared the frequency of AEs in communities receiving IA and azithromycin to that given IA followed by azithromycin MDA in Ethiopia [154]. In this study, the most reported AEs were headaches, gastrointestinal disturbances, and dizziness.

Furthermore, Campillo *et. al.* [59] conducted a systematic literature search and identified 10 cases of IVM-associated events reported by different authors in their review, including cutaneous reactions [155-159], nephropathy [160], psychiatric disorders [161, 162], hepatic disorders [85] , and multiorgan dysfunction syndrome [163]. In this review of Vigibase, Campillo *et. al.* further observed an increased reporting for toxidermias, encephalopathies, and confusional disorders after IVM use in SSA and other parts of the world. IVM is known to cause severe encephalopathies in those infected with loiasis, but this review identified serious

ADRs outside loiasis-endemic regions. Additionally, Twum-Danso presented a summary of reported cases of SAEs following treatment with IVM in oncho MDA through a passive surveillance system [164]. In this review, many SAE cases were reported giving rise to a cumulative incidence of one SAE per 800,000 reported treatments. In this review, approximately 50% of the reported cases were encephalopathic illnesses, of which 94% were reported from Cameroon. Many other studies have detected encephalopathy after administration of IA particularly in areas where Loa loa infection is common [165-171].

We did not systematically measure the incidence of encephalopathy in our study because loiasis is not prevalent in the Mkinga district. Cases of encephalopathy were also not suspected in our study. However, despite that, our focus was mainly on LF endemic communities without loiasis co-endemicity, and since encephalopathy after administration of IVM and ALB has been evident in other countries, it is prudent to be vigilant of the typical presentation of loiasis in Tanzania due to the increase of travels and migration from endemic areas. As of today, no mapping studies have been done in Tanzania to determine the prevalence or spatial orientation of loiasis. The disease is restricted to the equatorial rainforest regions of Central and West Africa [172].

Conversely, our study was large enough and included 9,640 individuals out of 10,000 targeted before in the sample size calculation. Lost to follow-up was only 3.7% (n=352). The large sample size enabled us to detect rare severe AEs, and the seven-day follow-up period allowed us to identify the time-curse as recommended by WHO. The study was powered to identify AEs, and risk factors and determine the incidence rate of occurrence of events in the population. A significantly higher incidence of AEs among patients with chronic LF manifestations than those without was observed. Higher incidence rates of AEs amongst females were as well detected as compared to males. This was attributed to both sex and gender-related factors. Chronic illnesses, in particular hypertension and asthma, were also significant risk factors for AEs. The frequency of AEs including fever, dizziness, stomach pain, diarrhoea, breathing difficulty, vomiting, and confusion was mostly seen in hypertensive patients. An association between venous hypertension and lymphoedema has been seen in several studies conducted elsewhere [173-177]. This association was further reported by Vaqas and Ryan to be due to capillary filtration and inflammation [178] which is mostly seen in individuals with chronic LF. Headache, dizziness, loss of appetite, difficulty in breathing, and vomiting were AEs mostly observed in those with asthma. The association of asthma with AEs in LF endemic areas had been reported to be due to Tropical Pulmonary Eosinophilia (TPE). TPE is a pulmonary syndrome that occurs in response to trapped microfilariae within the lung tissue (an exaggerated immune response to the filarial antigens) [179]. It is characterized

by nocturnal cough, dyspnoea, and wheezing with eosinophils playing a pivotal role [179, 180]. Eosinophilia was also frequently reported in individuals using IVM in the review of Vigibase [59]. Many other studies have reported the association between TPE and filariasis [181-189].

Alternatively, we did not find any significant association of AEs with age groups, the use of traditional medicines, or the number of IVM tablets taken in our study. Other risk factors such as kidney disease, diabetes, and TB were also not associated with AEs. The incidence of AEs between mf and CFA positives versus healthy individuals was also not investigated. We similarly did not assess the effects of MDA drugs in pregnancy as pregnant women and children below five years of age were excluded from the study because IA combination is contraindicated in these groups. Pregnant women are excluded based on their last menses [190]. On the flip side, Ndyomugyenyi R *et. al* conducted a study in Uganda and did not observe severe AEs during the second trimester of pregnancy after the administration of IVM, ALB, or a combination of both [191]. Despite this positive finding, investigators recommended long-term follow-up of pregnant women to assess safety in case of inadvertent use of the drugs that can happen in remote areas where behavioural and diagnostic techniques to detect early pregnancy are sometimes missing. As suggested by Gyapong [190], women should be asked to come for their MDA drugs only after they have had some evidence of their menses for that particular month. The NTD programme must therefore be extra vigilant in future MDA campaigns in the event of inadvertent exposure of pregnant women to IA in early pregnancy.

Our study was the first large-scale CEM study to actively investigate the incidence and associated risk factors of AEs following MDA of IA in Tanzania and SSA. The study enabled us to quantify the incidence and timing of each type of AE. It further provided relevant and adequate information on the safety of IA when used during MDA. CEM study design, which was adopted, is likewise key in estimating the incidence rate as compared to the spontaneous reporting system. The feasibility of conducting active safety surveillance in the MDA programme was demonstrated. CEM also proved to be pivotal in improving under-reporting particularly in resource-limited settings. The same can be replicated and adopted by the NTD programme in future MDA programmes. A close collaboration between PHPs and NMRAs is critical in integrating PV during MDA campaigns and in practice. The TMDA as the regulatory authority and NTD programme in Tanzania should continue to collaborate on safety monitoring during future MDA campaigns for timely detection, understanding, joint assessment, and prevention of AEs.

5.3 Surveillance of Haematological and Biochemical Parameters Following MDA (Paper III)

Haematological and biochemical parameters are commonly not measured during MDA. In this project, we investigated changes in these parameters following MDA among individuals residing in LF-endemic areas in Tanzania (***Paper III***). The aim was to identify whether LF infection status influences haematological and biochemical changes following MDA with IA. In this study, the median (IQR) values of laboratory parameters before and after MDA were compared to determine any change after drug intake. The comparison of proportions of individuals with abnormal parameters before and after MDA was further conducted.

The overall findings indicated the occurrence of changes in some laboratory parameters after IA intake. The Wilcoxon Signed Rank test revealed that the median values of the haematological parameters - RBC, Hb, and HCT decreased, while MCH, MCHC, monocyte, and eosinophil levels increased significantly following MDA. Platelet counts, including MPV and PLCR, increased in both CFA-positive and negative groups. Biochemical parameters, including BilT and BilD, decreased in the CFA-positive individuals and increased in the CFA-negative individuals. AST increased in the CFA-positive group while ALT decreased in the CFA-negative individuals. The McNemar test likewise revealed a significant proportion of individuals with abnormal parameters after drug intake.

5.3.1 Change in haematological parameters after MDA

Blood is widely used in toxicological research including safety studies as an indicator of physiological and pathological changes [192] The haematological parameters - Hb, HCT, RBC, WBC, and haematological indices such as MCV, MCH, and MCHC are commonly examined to assess the toxic stress induced by drugs and other pollutants [192]. Alterations of these parameters are considered as an anaemic condition in humans and animals. The decrease in RBCs, Hb, and HCT content usually reflects the harmful effects of drugs on the carrying capacity of oxygen in the blood. They are commonly used to diagnose anaemia [193]. A potential decline of these parameters demonstrates a potential risk for anaemia among individuals exposed to drugs. The median values of RBC, Hb, and HCT were below the normal baseline range after MDA in a significant number of study participants in our study. The values decreased significantly following IA intake regardless of CFA status.

The logistic regression performed did not reveal any predictor of low Hb concentration in males or females post-MDA. The logistic regression focused on Hb concentration since this is the critical surrogate marker for anaemia. It is known from the literature that females have a higher risk of experiencing low Hb as compared to males. Furthermore, despite that the baseline median Hb was

significantly higher in CFA positive group than in CFA negative, CFA status did not predict low Hb concentration after MDA, signifying that being CFA positive may not affect Hb levels.

Overall, many individuals had values within the reference ranges for MCH and MCHC post-MDA. MCH and MCHC measure the average amount of Hb in one or a group of RBCs. MCH and MCV may be significant in classifying anaemia into microcytic, macrocytic, or normocytic types [194]. Macrocytic anaemia occurs when the bone marrow produces abnormally large RBCs which lack nutrients [195].

Macrocytosis in adults is defined as an RBC mean MCV greater than 100 femtoliter (fL). The two most common forms of macrocytic anaemia are megaloblastic and non-megaloblastic macrocytic anaemias. Megaloblastic anaemia is caused by a deficiency or impaired utilization of vitamin B12 and/or folate, whereas non-megaloblastic macrocytic anaemia is caused by various diseases such as myelodysplastic syndrome (MDS), liver dysfunction, alcoholism, hypothyroidism, certain drugs, or inherited disorders of DNA synthesis [196-199]. Microcytic anaemia is defined as a reduced Hb synthesis associated with RBC MCV less than 80 fL during adulthood [200]. It is the most common form of anaemia, both in adulthood and in childhood. MCV is lower in childhood than in adulthood, and a nutritional iron deficiency may account for the appearance of congenital microcytic anaemia which might occur during or around birth and can be misdiagnosed as an inherited form of microcytic anaemia [201]. Microcytic anaemias are highly heterogeneous, and they may be either acquired (mostly due to iron deficiency) or inherited [202]. The median values of MCH and MCHC increased significantly following MDA in our study. However, they were within the normal clinical range post-MDA. In this respect, most individuals did not have macro or microcytosis.

On the other hand, changes in WBCs and its indices (lymphocytes, monocytes, neutrophils, and eosinophils) are considered as an excellent indicator for stress response. WBCs are mainly involved in the regulation of immunological defence functions against both foreign bodies and infectious diseases in humans [203]. The lymphocyte is the most dominant differential leukocyte and responsible for many functions of the immune system [203]. WBCs reduction may indicate immune suppression while increasing values indicate a response to stress or infection. The decrease of WBCs and lymphocytes might be due to the malfunctioning of the hematopoietic system [203].

In our study, there was no statistically significant change in the median values of WBCs before and after 7 days of receiving MDA. Alternatively, the median values for monocytes in the CFA-positive and negative groups increased significantly following MDA. A significant proportion of individuals was further observed to have

abnormal values from the reference range. A significant increase in median values for monocytes in COVID-19 patients treated with IVM was reported previously [204]. This was in tandem with what we observed after IA intake in our study.

IVM has also been reported to be associated with eosinophilia in the review of Vigibase. In our study, median levels of eosinophils increased in CFA-negative individuals. A significant proportion of individuals had as well shown abnormal values from the reference range. Eosinophilia in patients with filariasis had also been reported to be caused by higher levels of WBCs in blood accompanied by high levels of immunoglobulin E (IgE) and antibodies against mf [181, 182, 205].

Additionally, our study also measured the platelet counts to investigate any associated thromboembolic disorders after IA intake post-MDA. Platelet markers embrace PDW, MPV, PLCR, and PCT. The PDW increases during platelet depletion when turnover is accelerated and shares a similar pattern of behaviour to MPV during acute severe infections. PLCR identifies the largest-sized fraction of platelets. An increase in PLCR usually signifies that there is an increase in new platelets (which are larger). PCT is the plateletcrit that is influenced by the number and size of platelets and has a positive relationship with the platelet count [206]. These platelet markers are surrogates for thromboembolic and bleeding disorders [207]. In our study, no significant change in PDW and PCT was observed except for MPV and PLCR which increased in both groups following MDA. However, following the McNemar test, a great proportion of individuals had significant PLT abnormality. We therefore recommended that further studies be conducted to assess the potential association of IA use with changes in platelet counts and consequently thromboembolic or bleeding disorders.

5.3.2 Change in biochemical parameters following MDA

Biochemical parameters including renal and liver function tests were monitored following MDA. Our findings revealed a change in median values for BilD and BilT, which remarkably decreased in the CFA-positive and increased in the CFA-negative groups, respectively. Similarly, median values of AST increased in the CFA-positive individuals while those for ALT decreased in the CFA-negative group. A significant proportion of individuals with abnormal AST levels was again noted following MDA. Even further, the median values of creatinine increased in the CFA-positive group and many individuals had values lower than the reference range.

Principally, high ALT levels denote liver cell damage when the liver is injured. Higher than normal creatinine or bilirubin levels also indicate different types of kidney, liver or bile duct disorders. In clinical practice, the elevation of AST is also a sign of liver damage or damage to other organs including the heart, kidney, or skeletal

muscles. Our findings suggested that IA may be associated with biochemical changes, and we recommended further studies to explore this observation.

A single case of apparent liver injury was reported after IVM use in a study done in Cameroon. In this case, IVM was found to be associated with a low rate of serum ALT elevations. The onset of injury occurred one month after a single dose and was characterized by a hepatocellular pattern of serum enzyme elevations without jaundice [85]. Nonetheless, serum bilirubin and ALP levels were normal in this study.

Oscanoa et. al. reviewed reports of serious hepatic disorders associated with the use of IVM for COVID-19 patients in the Vigibase. In their review, they discovered that 6 individuals out of 25 who reported serious ADRs after IVM use, experienced hepatic disorders (i.e., hepatitis, hepatocellular injury, cholestasis, increased ALT and/or ASP levels, and other liver abnormalities) [208]. Nonetheless, a study done by (Kanjo Shiynsa F, 2021) and (Ozer et al., 2022) revealed no significant association between elevated levels of ALT and AST after repeated use of IVM in patients with Oncho and COVID-19 [86, 209]. However, the same study by Kanjo Shiynsa F, 2021 also reported significant changes in serum creatinine and blood urea levels. Alternatively, a systematic PV review done by Campillo et. al. reported no serious liver damage associated with IVM use in SSA [59].

Our results denoted changes in haematological and biochemical parameters after IA intake and I recommend more studies to be done to investigate this effect further. The pharmacovigilance system must be up to the task of detecting any abnormalities of the liver or kidneys as these vital organs have the potential risk of harmful and detrimental effects to human beings once they are affected.

5.4 Pharmacokinetics Analysis of IVM Following MDA (Paper IV)

In ***Paper IV*** we conducted a PK study to investigate the disposition of IVM alone in a population at risk of LF in Mkinga district. This was done because the disposition kinetics of IVM is an important determinant of its safety as well as efficacy. Our further literature search also revealed that data regarding the PK of IVM in humans are scarce as most studies were conducted in animals and a low number of healthy adults or adults infected with *Onchocerca volvulus* or *Plasmodium falciparum* malaria [62, 92-96]. Apart from the disposition of IVM we also identified predictors of its PK following MDA.

5.4.1 Initial PK study

In the initial PK study, 468 individuals residing in Mkinga district were sampled and their blood drawn for PK analysis. The initial intention was to measure all PK parameters including Cmax, Tmax and AUC. Considering the invasive nature of blood sampling including taking into account the elimination half-life of IVM which

ranges between 25 and 80 hours [98, 99, 103], it was decided to compute a POPPK model to estimate the $AUC_{0-\infty}$ and elimination half-life and measure bioavailability of the drug. The model was therefore searched, validated, and adopted to obtain the intended PK parameters and explore the predictors of IVM disposition using 4 sampling time points (i.e., at 0-, 2-, 4-, and 6-hours).

5.4.2 POPPK analysis

The POPPK analysis was developed according to a two-compartment model, first-order absorption and linear disposition kinetics as previously reported [111]. In model simulations, the predictability of the model can be evaluated by comparing the model prediction values with the observed ones not used in the construction of the model. The developed POPPK model provided a good description of IVM observed data. The goodness-of-fit criteria showed evidence that the final model was consistent with the observed data and that no bias was recorded. The weighted residuals were homogenous and randomly distributed. Both fixed-effects and random-effects of the final model reflected the true observed data. The predictive criteria indicated that less than 5% of the data were located outside the 5^{th} to 95^{th} quantile range suggesting that the model accurately described the central tendency and the variability of the data. The comparable observed and model-estimated secondary PK parameters further supported the good agreement between the modelled and observed data.

5.4.2.1 PK parameters measurement

The observed and model-simulated $AUC_{0-\infty}$ and Cmax were comparable for each dose strata. An overall decreasing trend in $AUC_{0-\infty}$ but an increasing trend in Cmax from lower doses (3mg) to higher doses (12mg) was observed when comparing the systemic bioavailability. The estimated mean $AUC_{0-\infty}$ was relatively higher than that reported in the previous study [111]. Moderate to heavy *W. bancrofti* infection did not affect PK parameters for IVM, DEC, or ALB following a single co-administered dose of these drugs compared to uninfected individuals [210]. In this study, there was no difference in $AUC_{0-\infty}$ or Cmax between LF-infected and uninfected individuals ($P>0.05$ for all comparisons). The study evaluated the PK, safety, and efficacy of triple-drug therapy in people with and without *Wuchereria bancrofti* infection in West Africa. A mean Tmax of 4 hours both in observed and model-simulated data in our study was consistent with previous studies [111]. The apparent volume of distribution was higher in our study as compared to that of Duthaler *et al* [111]. However, the values of CL/F were the same. A longer elimination half-life was also observed in our study which was higher in individuals who received lower doses than those who were on higher doses. Nonetheless, the half-life was within the range reported in other studies [98, 99, 103].

5.4.2.2 Predictors of IVM PK

Since IVM is highly lipophilic with high membrane permeability [104] and because its plasma exposure depends on the BMI of an individual [97], we tested various parameters in our model to identify predictors of its disposition. This was also possible as the non-linear mixed-effects model methodology used in POPPK studies allows for identifying and quantifying the effect of multiple factors (i.e. covariates) to further explain the variability in drug exposure and optimization of drug therapy [211-214]. In our study, weight and dose were identified as significant predictors of IVM PK amongst other parameters investigated. The assessment of the current height-based dosing revealed significant under-dosing of some individuals weighing <30kg, 61-70kg, 71-80kg, or >80kg. Moreover, height-based dosing resulted in relatively lower $AUC_{0-\infty}$ for individuals weighing >70kg compared to those weighing <70kg. However, only individuals weighing <30 kg were under-dosed when the dose was calculated based on weight (i.e., 200 µg/kg) and whole tablets rounded to the nearest number (3 mg per tablet).

A study conducted by Goss et. al. also reported that height-based dosing of IVM resulted in 27% of individuals receiving treatment doses below those recommended by weight dosing [43]. Significant influence of body weight on IVM kinetics was also observed in our study which was congruent to what had been reported before in other studies [111]. A recent study done by Alshehri *et al.* in Ivory Coast showed a linear PK behaviour of IVM amongst the simulated dosing groups with similar drug exposure based on sex. There was likewise a higher drug exposure with the 18 mg fixed-dose regimen of IVM than with the dose based on body weight at 200 µg/kg; despite that the increase in drug exposure was relatively small. Sex was a significant covariate on the peripheral volume of distribution in this study (Vp/F, 53% lower in men than in women) [215].

Shu and Okonkwo studied the use of height, physical appearance, and mid-upper arm circumference (MUAC) for calculating the dose of IVM when used for Oncho in Nigeria. Of the three methods compared, the MUAC-based method was found to be more convenient and corresponded closely to the dosing of IVM by weight. An adaptation to this method was advocated [216]. Alexander *et. al.* proposed further research on both height and physical appearance methods be done in different settings and populations to identify which one should become the method of choice during Oncho MDA [44]. All these covariates other than weight and dose were not significant predictors of IVM kinetics in our study. As the MDA programme uses height poles when dosing individuals, and since this has proven to under-dose individuals in different weight bands, we recommended in our study the use of weighing scales for precise estimation of the number of tablets and therefore weight-based dosing of IVM during MDA. Using POPPK modelling,

we identified a dosing strategy for IVM which can be adapted by the NTD programme in future MDA campaigns. The correct dosing will lead to intended drug exposure and consequently reduce the transmission of LF in populations at risk.

5.4.2.3 Genetic polymorphisms and their influence on IVM kinetics

Our study investigated the possibility of whether genetic polymorphisms might influence the variability in IVM drug exposure. IVM is a substrate for Cytochrome P450 (CYP) enzymes and multidrug resistance (MDR1) genes, and its PK could be impacted by polymorphisms associated with these genes [33, 104, 217]. Genetic variations in metabolizing enzymes and a transporter (ABCB1) had no significant influence on IVM PK when added to our model.

5.5 Efficacy of IA in Suppressing Transmission of LF (Paper V)

The efficacy of IA can be confirmed through the microfilaricidal effect of these drugs. Previous studies have observed that IA can clear mf in blood following MDA. However, due to the development of drug resistance, the likelihood of recrudescence, variations in the intensity of infection, genetic factors, and parasite clearance rates, ongoing research is recommended to further document the efficacy of drugs in different settings. A literature search indicates that IA had been studied before to determine its effect on clearing mf in blood. Many years have passed and since the disease is still endemic in affected communities, further studies are warranted to continue to prove the efficacy of such MDA drugs. We therefore conducted an efficacy study to establish the mf clearance rate soon after MDA and six months later (**Paper V**).

This study investigated the efficacy of IA after MDA in endemic communities of rural Tanzania. CFA and mf status levels in individuals were measured before MDA, seven days after, and six months later. Our overall findings indicated that IA administered as preventive chemotherapy effectively clears microfilaremia (90%) from the blood within seven days without rebound after six months. Nevertheless, IA is less effective (12.6%) in clearing circulating filarial antigenemia six months post-MDA.

The findings of our study were in tandem with results reported from other studies conducted elsewhere [119-125, 218-222]. In our same study, drug exposure levels were determined using PK parameters to include Cmax and AUC. Such parameters were deduced from our previous study for IVM (**Paper IV**). The intention was to correlate IVM exposure with mf and CFA clearance. Generally, no significant correlation of variability in IVM plasma exposure with microfilariae and CFA clearance was observed. The minimum inter-individual variability as measured by CV was at ≤20%.

On the other hand, CFA clearance was remarkably low (12.6%) after six months post-MDA. From this observation, it was evident that IA has less effect on CFA clearance over time. The levels of antigenemia did not decline after six months of follow-up.

It can be postulated that the persistence of adult worms in the lymphatics for a long time, explains the current prevalence status (**Paper I**) and consequently the transmission of the disease in endemic communities. Since when MDA was introduced in Tanzania, still, the prevalence of infection has not gone down beyond the levels where recrudescence is unlikely to occur. More efforts are needed to eliminate the disease. And in this case, repeated MDA rounds are critical and should continue to interrupt disease transmission in endemic regions.

Combinations containing DEC should be sought as the drug can kill adult worms. Such combinations had shown superior efficacy in sustaining clearance of mf and will have large impact on LF [70, 223-226]. Effective MDA coverage with DEC containing regimens could accelerate the elimination of LF as a public health problem. The only challenge is that DEC-containing regimens are contraindicated in areas where LF is co-endemic with oncho and loiasis [5, 41]. The use of DEC in such areas has proven to be associated with the Mazzotti reaction which is life-threatening and fatal. Considering the benefits, it is therefore recommended that the NTD programme should re-map the co-endemicity of LF and Oncho to allow for DEC to be introduced in Tanzania.

6 Conclusions

The overall findings of the project can be summarized as follows:

- The prevalence of antigenemia positivity was 5.8% and that for microfilaraemia amongst those who tested positive for antigenemia was 5.2%. Due to this high prevalence, recrudescence is likely to occur and therefore Pre-TAS and subsequent TAS cannot be initiated in the studied district.
- Coverage of MDA in the previous year was measured at 60%. This means about 40% did not take MDA drugs in the previous year (i.e., 2017). Missing MDA was also significantly associated with a high risk of CFA-positivity. Low MDA coverage reduces the impact of MDA on transmission and delay in reaching the elimination targets.
- Two at-risk age groups (25–34 years and 65+ years old) who mostly missed MDA in the previous year had the highest infection rate. These age groups may serve as reservoirs of infection for continued transmission, hence the need for targeted intensive control measures.
- Non-compliance with MDA represents a serious programmatic challenge for the LF programme because systematically non-compliant individuals serve as a reservoir for the parasite and thus recrudescence of infection.
- Swelling of arms or legs was observed at a lower frequency and these were mainly in individuals with negative CFA tests. There were reduced hydrocele and lymphoedema rates in the young age group compared to the older group. Since lymphoedema and hydrocele are the most frequent sequelae observed in endemic communities, the NTD programme should continue monitoring and offering services to those suffering from the clinical manifestations of LF considering their social-economic status.
- IA combination preventive chemotherapy is generally safe and tolerable. Treatment-associated AEs are mild-to-moderate and transient, resolving within a week of MDA. Most AEs that occurred during the first two days of MDA were transient and resolved progressively to day seven. Headache, drowsiness, fever, dizziness, and stomach pain were the most common AEs. Risk factors that can predict the safety of IA include female sex, having pre-MDA clinical symptoms, chronic illnesses, in particular hypertension or asthma, and chronic manifestations of LF.
- CEM study design is pivotal in increasing the number of AE reports and the same should be adopted in future MDA campaigns and other safety monitoring programmes.

- Haematological and biochemical changes may occur following MDA with IA. The observed abnormalities need further scrutiny to ascertain the safety of MDA drugs in future campaigns.
- A POPPK model can be used to describe IVM disposition kinetics in a large population exposed to MDA. The model can also be used in future monitoring of MDA programmes.
- As the MDA programme uses height poles when dosing individuals, and since this has proven to under-dose individuals in different weight bands, the use of weighing scales for precise estimation of the number of tablets and therefore weight-based dosing of IVM during MDA is recommended.
- IA combination chemotherapy is effective in clearing mf in blood following MDA. However, CFA clearance was low as levels did not significantly decline six months post-MDA. Furthermore, there was no significant correlation of variability in IVM plasma exposure with mf and CFA clearance. A minimum inter-individual variability as measured by CV was observed.
- Persistence of adult worms in the lymphatics for a long time, explains the current prevalence status of LF in the studied district. Studies need to be done to re-map the co-endemicity of LF and Oncho to allow for DEC to be introduced in Tanzania.

7 Points of Perspective

Points of perspective that need to be considered by policymakers to include the NTDCP, National Regulatory Authorities (NRAs), and others are summarized below:

- The NTDCP needs to strengthen control measures to reduce the prevalence of the disease in the country. Despite that levels of CFA positivity have gone down quite considerably, but they are still above the threshold recommended by WHO for pre-TAS and TAS.
- The low MDA coverage in the district studied as compared to WHO recommendations of 65% coverage in an implementation unit, needs to be investigated and deliberate actions taken to change this status quo. The finding of infection hotspots with signs of ongoing transmission, due to non-adherence to MDA, calls for targeted intensive control measures. This was only one district that was studied and in case many other districts were studied, the situation could have been different. Consented efforts and measures to include continuous advocacy and education of communities are critical to entice individuals to take part in future MDA campaigns to increase coverage.
- The high-risk group for mosquito exposure (i.e., those with 25–34 years and 65+ old), should be taken into account in future MDA campaigns.
- The WHO GPELF target of eliminating LF as a public health problem by 2020 has not been attained. It is understood that a new target of 2030 has been set by WHO. The NTDCP must work hard together with other partners to ensure that this revised target is attained.
- Pharmacovigilance needs to be integrated into public health programmes. Since MDA drugs are distributed to all at-risk populations without a prior diagnosis, NRAs must work in close collaboration with NTDCPs to collect data on the safety of medicines deployed during MDA. This will increase reporting rates and help to detect new safety signals with the overall intention of protecting public health.
- Safety follow-up of individuals with underlying clinical conditions during MDA needs to be considered particularly in those over 65 years of age. This should also take into account the monitoring of haematological and biochemical surrogate markers after drug use.
- The effect of IA on haematological and biochemical markers needs to be further investigated in future studies.
- POPPK modelling can be applied to monitor drug disposition kinetics in future MDA programmes.

- Weight-based scaling should be used whenever possible during MDA rather than height-based measurements to allow for precise estimation of body weight and correct dosing of individuals.
- Since it is not clear for how long antigenemia subsides, more studies need to be done to determine the duration of CFA clearance post six months of MDA.
- Re-mapping of LF and Oncho co-endemicity is recommended to allow for DEC to be introduced in Tanzania as the drug has an effect on adult worms which are responsible for the persistence of LF infection.

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