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Pharmacokinetics of rifampicin and isoniazid in a Peruvian population under tuberculosis treatment

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TESIS DOCTORAL

PHARMACOKINETICS OF RIFAMPICIN AND ISONIAZID IN A PERUVIAN POPULATION UNDER TUBERCULOSIS TREATMENT

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Science knows no country, because knowledge belongs to humanity, and is the torch which illuminates the world.

Louis Pasteur

… to can Riquena

Tesis depositada por **Ana Requena-Méndez**, licenciada en Medicina y Cirugía, para optar al grado de Doctora en Medicina por la Universidad de Barcelona, bajo la dirección del Prof. David AJ Moore y el Dr. José Muñoz Gutiérrez.

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LIST OF PUBLICATIONS ARISING FROM THESIS WORK

Article 1

Pharmacokinetics of rifampin in Peruvian tuberculosis patients with and without comorbid diabetes or HIV.

Requena-Méndez A, Davies G, Ardrey A, Jave O, López-Romero SL, Ward SA, Moore DA.

Antimicrob Agents Chemother. 2012 May;56(5):2357-63. doi: 10.1128/AAC.06059-11. Epub 2012 Feb 13.

Article 2

Effects of dosage, comorbidities, and food on isoniazid pharmacokinetics in Peruvian tuberculosis patients.

Requena-Méndez A, Davies G, Waterhouse D, Ardrey A, Jave O, López-Romero SL, Ward SA, Moore DA.

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Article 3

Reply to "adequate design of pharmacokinetic-pharmacodynamic studies will help optimize tuberculosis treatment for the future".

Requena-Méndez A, Davies G, Moore DA.

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Article 4

Robust and Reproducible Quantification of the Extent of Chest Radiographic Abnormalities (And It's Free!).

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Article 5

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Requena-Méndez A, Davies G, Waterhouse D, Lopez-Romero S, Ward S, Moore DA.

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GRANTS ARISING FROM THE THESIS WORK

1. **Title of the project**: Pharmacokinetics of rifampicin and isoniazid in patients with tuberculosis and co-morbidities (DM or HIV) under tuberculosis treatment.
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GLOSSARY

This glossary has been taken from the “Suggested Language and usage for Tuberculosis (TB) care, communications and publications” [1] elaborated by the STOP-TB Partnership and from the “Definitions and reporting framework for tuberculosis-2013 revision” [2] elaborated by WHO.

Case: The definition of a TB case is currently under review by the World Health Organization. Although the term will doubtless continue to be used widely in public health, it should be used with sensitivity in health care settings to avoid dehumanizing patients. A person is not a case but a fellow human being. People seeking or receiving care may find it demeaning if they overhear a health worker describing them as ‘cases’.

Definite case of TB: This term is defined by having *Mycobacterium tuberculosis* complex identified from a clinical specimen, either by culture or by a newer method such as Xpert MTB/RIF or molecular line probe assay. In countries that lack the laboratory capacity to routinely identify *M. tuberculosis*, pulmonary TB with one or more initial sputum smear examinations positive for acid-fast bacilli is also considered to be a “definite” case, provided that there is a functional external quality assurance system with blind rechecking.

Extrapulmonary TB: This term refers to TB involving organs other than the lungs, such as pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones or meninges. Diagnosis should be based on at least one specimen with confirmed *M. tuberculosis* or histological or strong clinical evidence consistent with active extrapulmonary TB, followed by a decision by a clinician to treat with a full course of TB chemotherapy. Unless a case of EPTB is confirmed by culture as caused by *M. tuberculosis*, it cannot meet the “definite case” definition (see page 4).

High-burden country: The term ‘high burden’ refers to one of the 22 countries that together have 80% of all new TB cases arising each year. This expression should be used with caution and sensitivity in order to avoid stigmatization

Multidrug-resistant tuberculosis (MDR-TB): MDR-TB is a specific form of drug-resistant TB, due to bacilli resistant to at least isoniazid and rifampicin, the two most powerful anti-TB drugs.

Millennium Development Goals (MDGs): Eight goals were agreed at the Millennium Summit in September 2000. Goal 6 refers specifically to halting and reversing HIV and

to halting and reversing the incidence of malaria and other major diseases, with specific mention of TB, by 2015. Lack of progress across other MDGs may seriously curtail progress in tackling TB and, conversely, success in attaining other MDGs is being hampered by the TB epidemic. See http://www.un.org/millenniumgoals/aids.shtml.

Extensively drug-resistant TB (XDR-TB): This is a form of drug-resistant TB in which bacteria are resistant to isoniazid and rifampicin, the two most powerful anti-TB drugs, plus fluoroquinolones and at least one injectable second-line drug.

Cured: A pulmonary TB patient with bacteriologically confirmed TB at the beginning of treatment who was smear- or culture-negative in the last month of treatment and on at least one previous occasion.

Treatment completed: A TB patient who completed treatment without evidence of failure but with no record to show that sputum smear or culture results in the last month of treatment and on at least one previous occasion were negative, either because tests were not done or because results are unavailable.

Death: A TB patient who dies for any reason before starting or during the course of treatment.

Treatment failure: A TB patient whose sputum smear or culture is positive at month 5 or later during treatment

Early relapse: Patient who has previously been treated for TB, was declared cured or treatment completed at the end of their most recent course of treatment, and within 6 months is diagnosed with a recurrent episode of TB (either a true relapse or a new episode of TB caused by reinfection).

Lost to follow-up: A person lost to follow-up is a person who meets the following criteria. I. someone whose diagnosis of TB disease has been confirmed, but who does not appear in the TB patient register and is therefore not registered as having started treatment. In practice, such a person has sought care from health services, and has been diagnosed with TB, but does not end up being registered for treatment. II. A person who began TB treatment that is interrupted for two consecutive months or

more. III. A patient who is declared as having interrupted TB treatment for two months or more, and then returns to the TB services.

Smear-positive pulmonary TB: Sputum smear-positive pulmonary TB is defined as the presence of at least one acid fast bacillus in at least one sputum sample in countries with a well-functioning external quality assurance system.

Abbreviations and Acronyms

ART: Antiretroviral therapy.
AUC: Area under the curve.
BMI: Body Mass Index
Cmax: Peak serum drug concentration
DM: Diabetes Mellitus
DOT: Directly observed treatment
HPLC: High-performance liquid chromatography
MDR-TB: Multidrug resistant TB
MDG: Millennium Development Goal
MODS: Microsocopic observation drug susceptibility
PNTP: Peruvian National TB program
PK: Pharmacokinetics
TB Tuberculosis
TDM: Therapeutic drug monitoring.
Tmax: Time at which Cmax occurs.
WHO: World Health Organization

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1. INTRODUCTION

1.1 TUBERCULOSIS: GENERAL ASPECTS

Tuberculosis (TB), an infectious disease caused by Mycobacterium tuberculosis, remains a major global health problem, being the second leading cause of death from an infectious disease worldwide [3]. In 2014, it was estimated around 9.6 million new cases of TB and 1.5 million people died from TB. About one quarter of those who dies were HIV-positive [4]. Global statistics estimate that one-third of the population is infected with *Mycobacterium tuberculosis.* One out of every ten people infected will develop the disease.

Short-courses regimens of first line TB-drugs can cure around 90% of cases. Thus, TB mortality is unacceptable considering that most deaths are preventable if people can access to health care. However, drug resistance TB (DR-TB) poses a major threat to control TB worldwide[3].

The emergence of treatment failure and TB drug resistance was first described early after the administration of streptomycin. The situation has evolved with the appearance of multidrug resistant TB (MDR-TB) and subsequently, extensively drug resistant TB (XDR-TB)[4]. Innovative approaches and more funding to increase the uptake of programmatic management of DR-TB globally are urgently required to detect and enrol more patients on MDR-TB treatment, and to improve health outcomes. In parallel, more efforts are needed to identify possible individual and social determinants of this disease. In fact, it is unclear if the health impacts of TB are irrespective of the socio-economic status though there are some vulnerable groups –i.e. the poor, migrants, persons living in urban marginalized areas, prisoners, people with HIV/AIDS, and indigenous populations - which present the major burden of disease.

The current TB strategy focuses on prompt diagnosis and treatment, which is aimed at reducing the transmission of the pathogen [3]. The laboratory confirmation of these TB cases as well as a progression in the detection of MDR-TB, facilitated by the use of rapid diagnostic tests, is key to ensuring that individuals are correctly diagnosed and treated.

The 2015 Millennium Development Goal (MDG) of halting and reversing TB incidence has been globally achieved in all six WHO regions, and that includes most of the 22 high TB burden countries (HBCs)[5]. TB worldwide incidence fell at an average rate of about 1.5% per year between 2000 and 2013. Globally, the TB mortality rate fell by 45% between 1990 and 2013. However, 3.5% of new and 20.5% of previously treated cases were estimated to have multi-drug resistant TB (MDR-TB). These percentages

have not decreased compared with recent years and indeed they are a major concern in some parts of the world.

The end of 2015 will mark the transition from the MDG to a post-2015 global TB strategy which has been developed by WHO and was already approved by all member states of WHO at the May 2014 World Health Assembly[6]. The new goal will be to end the global TB epidemic, targeting a 95% reduction in TB deaths, 90% reduction in TB incidence and a “zero” catastrophic costs for TB-affected families by 2020[6]. Major efforts are needed to ensure all cases are detected, notified and treated[3].

1.2 THE CONTEXT OF TUBERCULOSIS IN PERU

In the last decades, the strengthening of the Peruvian national TB program, the universalization of the directly observed treatment (DOTs) program, and also the socio-economic development have achieved a reduction in the estimated incidence of TB per 100000 people from 317 in 1990[7] to 124 in 2013 in Peru [8]. A comprehensive and strengthened DOTS program which covers almost 100% of population has certainly been a substantial contributing factor. This was implemented based on the 5 components of the DOTS framework: (i)sustained political commitment, (ii)effective drug supply, (iii)monitoring and evaluation of performance and impact (iv)provision of standardized treatment through multidisciplinary team from community leaders to health workers and DOTS volunteers and (v)early case detection and diagnosis[9,10]. In parallel, by 2005 a successful decrease in the percentage of treatment failure, abandonment of treatment and mortality was observed [11]. Despite this encouraging evidence in the reduction of incidence, morbidity and mortality, the Andean country of Peru has one of the highest annual incidence rates (See figure 1), mortality rate and drug resistance rate of TB, particularly MDR-TB in the Americas. One study that evaluated all cause and cause-specific of death among older people, showed that tuberculosis was the leading cause of death among the rural population and was also one of the five leading cause-of death among the urban population [12].

Although Peru accounts for only 3% of the population of the Americas, around 12% of this region’s TB patients reside in Peru, and 32% of MDR-TB patients are also from Peru[13]. According to the last WHO report, in 2013 Peru had an incidence of 124/100000 habitants and a TB notification rates of 79 cases per 100,000 habitants, with an 3.9% of new TB cases with MDR-TB[8]. Moreover, prevalent latent infection persists in the adult population despite the socio-economic development and improving medical care[14].

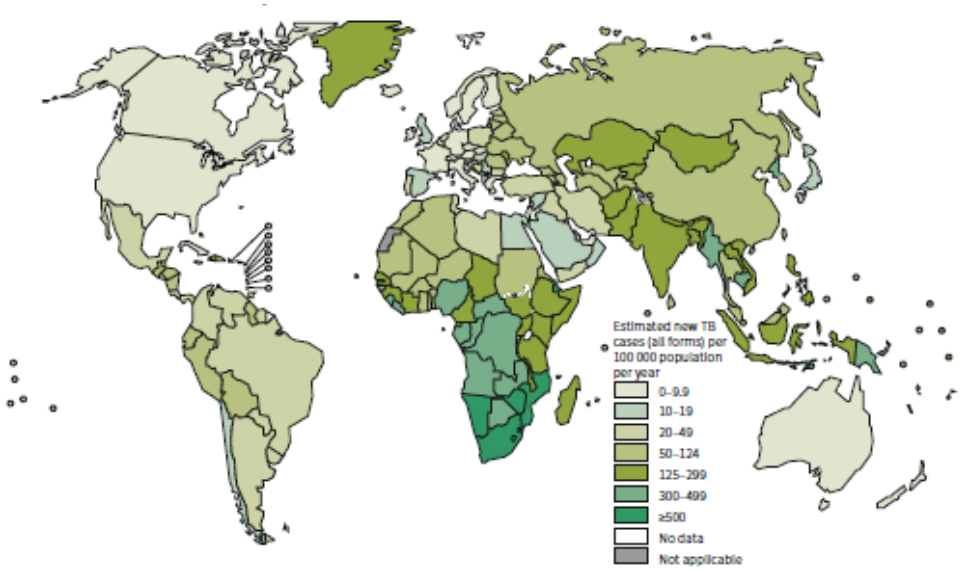


Figure 1 Estimated TB incidence rates, 2013. Source: Global Tuberculosis report. 2014-WHO

A WHO report stated, regarding drug-resistance of TB, that even though Peru is not included in the list of the 27 MDR-TB high burden countries, in 2008 it was the country in the Americas with the highest absolute number of MDR-TB cases (2600), and also the highest proportions (5,3%) of MDR-TB cases among new cases in the Americas following Dominican Republic [13].

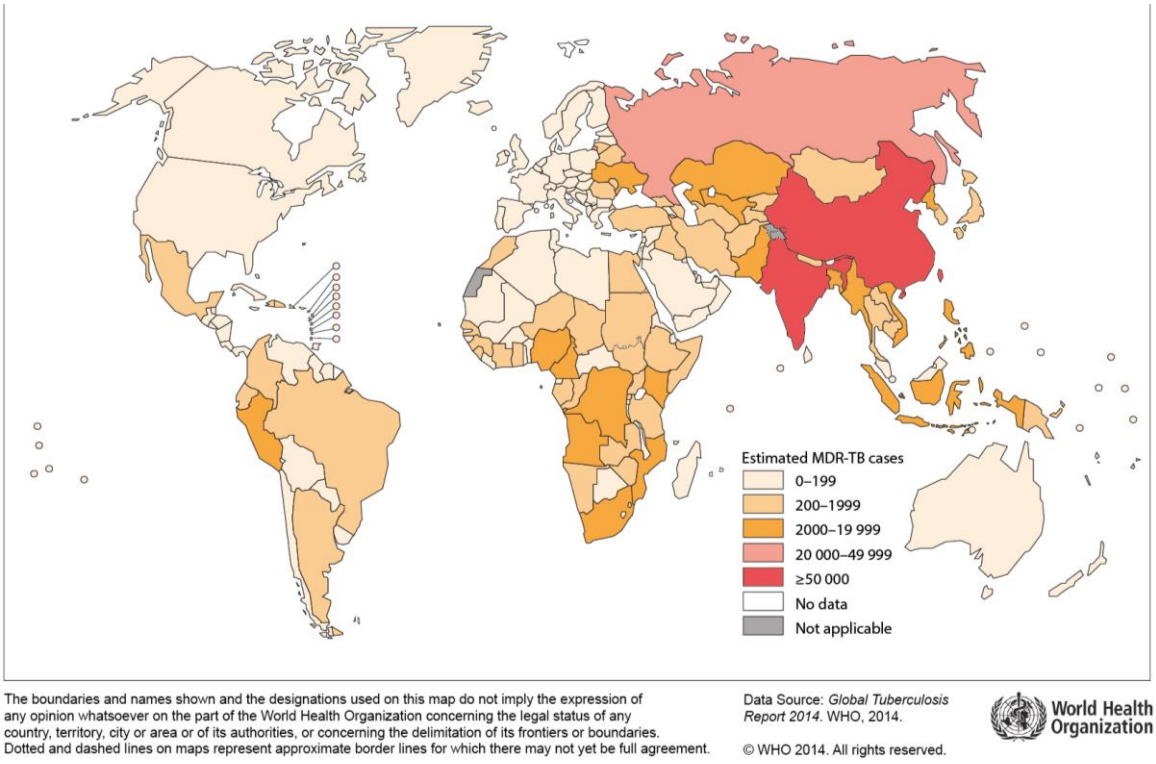


Figure 2 Number of MDR-TB cases estimated to occur among notified pulmonary TB cases, 2013. Data Source: Global Tuberculosis Report 2014. WHO, 2014

In 2007, the regions with more TB cases rate were Lima, Callao, Ica, Tacna, Madre de Dios, Ucayali and Loreto[15]. In particular, Lima and Callao are reporting the majority of cases with 58% of all cases of TB, 82% of MDR-TB and 93% of XDR-TB cases[15,16]. These cities have an overwhelming risk of active transmission[17]. As shown in Figure 3, the absolute number of cases reported in these two Peruvian provinces exceeds the number of cases in all American countries, excepting Brazil and Mexico, both with larger populations. It can also be seen that one of the health sectors of Lima, "Lima Sur", (which covers the districts of Barranco, Chorrillos, Surco, San Juan de Lurigancho, Villa María Triunfo and Villa Salvador) present more cases of TB than countries like Chile, Paraguay, Nicaragua or Canada[16].

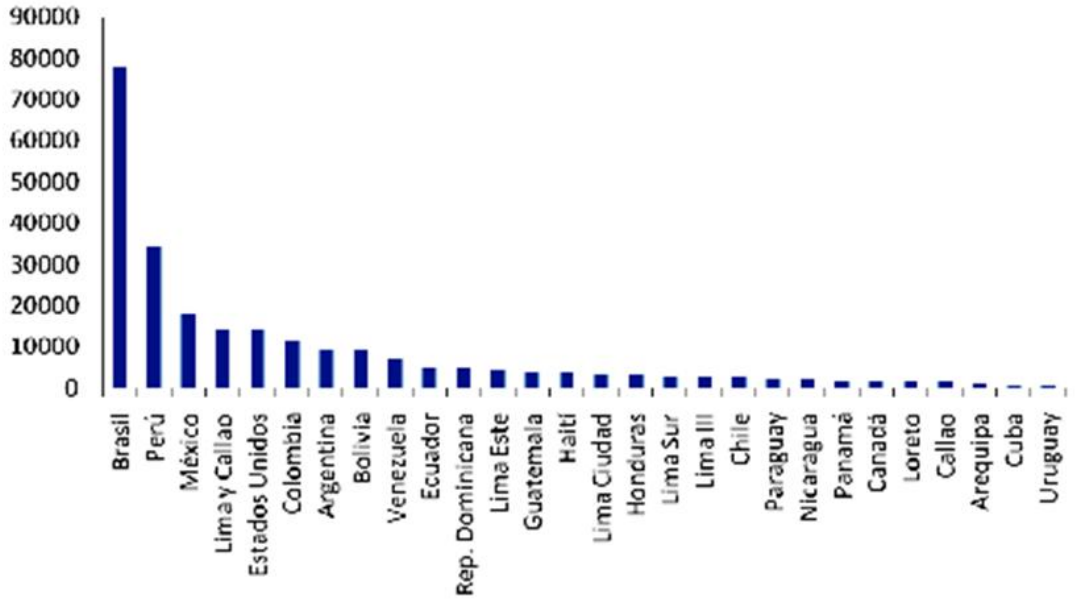


Figure 3 Absolute number of TB cases notified by American countries and departments in Peru. Source: Situación actual y propuesta de lineamiento técnicos para el control de la tuberculosis resistente, Perú, 2008

At present, TB is still a major Public Health concern and major efforts are currently being focused on enhancing institutional capacity of regional counterparts and on dealing with the drug resistance issue.

1.3 FACTORS ASSOCIATED WITH POOR TREATMENT OUTCOME

The current first-line strategy to treat tuberculosis (TB) is based on the standardized short course regimens recommended by WHO, including rifampicin, isoniazid,

pyrazinamide and ethambutol which are usually highly effective[18] and have few side effects[19]. Poor compliance with treatment is the major cause of treatment failure and relapse. Accordingly, adherence to the treatment has to be maintained which has been improved through the directly observed therapy (DOT) strategies[18,20]. Despite the success of these programmes in many TB endemic countries, relapse and acquired drug resistance has not been entirely eliminated[21]. In this sense, other factors such as high bacillary burden or sputum smear- positivity, cavitation, co-morbidities such as HIV or DM and other underlying diseases have been clearly associated with poorer TB outcome[22–25].

1.3.1 Diabetes Mellitus

Diabetes Mellitus (DM) is one of the major non-communicable diseases and, global statistics estimate an increase from 194 million people with this disease in 2003 to 330 million in 2030[26,27]. A substantial percentage of this (75%) will be living in developing countries[28].

Many researchers have largely reported an association between DM and TB. It was firstly documented by Avicenna in the 11th Century b.c.[29]. It is currently estimated that 10% of TB cases are associated with DM[30] and a meta-analysis showed that TB and DM are indeed positively associated (RR: 3.1, CI: 2.3-4.3)[31]. DM not only clearly increases the risk of TB[31–33] but also increases the risk of having more pulmonary TB (PTB) with higher proportion of sputum smear positivity and also of having an unfavourable outcome (treatment failure or death) and these patients are also more likely to relapse[33,34]. Therefore, increases in TB prevalence and incidence over time are more likely to occur when diabetes prevalence also increases[35].

Finally, some studies have reported an independent association between DM and MDR-TB[36,37] although data are limited regarding possible risk factors associated to MDR-TB and DM[38].

In this sense, it is imperative to gain an adequate understanding of this association, especially since 8 of the top 10 countries with the highest incidence of DM in the world also present a major burden of TB according to WHO reports[30]. In particular, it is expected that in 20 years, 75% of diabetics will be living in countries where the burden of TB will be very high[26].

For all these reasons, there is a need to perform early bi-directional screening programmes and explore the underlying mechanism for different treatment outcomes for PTB with DM.

There are some countries of particular concern, and Peru is included in this list, given their size, TB burden and large projected increases in DM prevalence[35]. In Peru, the prevalence of diabetes in urban areas was reported to be 7%[39]; based on limited data, the national prevalence is estimated to be 5.1–6.0% among adults and is expected to increase to 7.3% by 2030[38]. There are limited data about the association of TB-DM in Peru. In a Peruvian cohort of 1671 TB patients at higher risk of having drug resistance, around 11% had DM [38]. A descriptive study of DM and TB patients in Peru illustrated an adult and predominantly male population, with poor glycaemic control. It showed also a higher number of treatment failures, relapses and acquired MDR-TB among diabetics, suggesting the possibility of nosocomial MDR-TB transmission as the cause of this fact[40]. Both studies have considerable limitations that could have contributed to selection bias. So, there is a lack of information about this association, not only from the epidemiological point of view but also from a clinical perspective.

1.3.2 TB-HIV

The HIV/AIDS pandemic is still one of the major cause of mortality among infectious diseases in the entire world despite the scaling-up of antiretroviral therapy (ART) in recent years in developing countries[41]. Between 46,000 and 96000 people were estimated to be HIV positive in Peru in 2013 and around 2800 people were dying because of HIV according to last WHO estimates[42]. More than 32,000 AIDS cases have been notified in Peru throughout the evolution of this epidemic [43]. Figure 4 shows the cumulative notified cases in Peru from 1983 to 2013 by department. The global incidence in 2014 was 3.14 per 100000 habitants[43].

It must be said that the global prevalence within the country does not exceed 0.5%. The epidemic predominantly affects men who have sex with men (MSM), with higher prevalence in urban cities like Lima, particularly in the coastline and rainforest regions [44]. However, the epidemic is slowly spreading to the rural population, where it is having a severe impact on vulnerable groups (e.g. the poorest and women) [44].

There is little doubt that TB is strongly associated with HIV, as it is a major cause of morbidity and mortality in HIV patients in low-income countries [45]. HIV may increase susceptibility to TB infection; it increases the risk of reactivating latent TB infection and

the TB progression after either infection or reinfection with MTB, particularly in immunosuppressed patients[46–48]. TB incidence rose rapidly in those countries with high rates of HIV/AIDS infection such as those in eastern and southern Africa [49].

Figure 4 Cumulative cases from 1983-2013. Source: Boletin VIH/SIDA. MINSA-Peru

In 2013, there were an estimated 1.1 million HIV positive new TB cases[50]. Moreover, some studies have suggested that HIV-positive sero-status could be considered a risk factor for resistance to at least isoniazid, or rifampicin or both [51].

According to relevant health data in Peru, 40% of HIV-positive patients hospitalized at Hospital Nacional Dos de Mayo had TB[52] and 43% of these were MDR-TB[53]. Conversely, 2.5% of all TB patients and 3.4% of MDR-TB patients were HIV positive[54]. Other studies have also supported the fact that co-infection of TB with HIV is associated with early mortality, especially in multidrug resistant TB (MDR-TB) patients in Peru, one of the countries with the highest burden of MDR-TB[55].

1.4 PHARMACOKINETICS OF TB DRUGS

Most TB patients are treated with a standard dose of drugs and they generally exhibit a high cure rate with no significant side effects[19]. Poor compliance to treatment has been reported as the major cause of treatment failure and relapse. Nevertheless, an unknown percentage with a poor response to treatment (defined by clinical failure or relapse) could be due to pharmacokinetic impairment (low drug concentration) of

antituberculosis drugs according to in-vitro, animal and in-vivo data[56–60]. Moreover, in the last years, experimental and clinical studies have shown that pharmacokinetic variability expressed in key parameters such as plasma area-under-the-curve (AUC) seems to play a more important role in the emergence of acquired multidrug-resistant TB (MDR-TB) in *in vitro* models since inadequate exposure to anti-TB drugs has been associated with acquired drug resistance [61,62]. In clinical prospective studies, impaired pharmacokinetic studies have been also related to a suboptimal treatment response[63,64] but this fact could not be demonstrated in other studies[57,65,66]. However, the number of studies assessing the relation between plasma concentration and treatment outcome are scarce and the majority of studies evaluated this association considering a separate anti-TB drug instead of the combination therapy, which remains a limitation.

The pharmacokinetic threshold of TB drugs in drug-susceptible patients below which treatment outcome is expected to be poorer have been already described[67]. Based on these categorization of the antituberculosis drug PKs, it has been suggested that therapeutic drug monitoring (TDM) can be used to optimize dosing that maximizes therapeutic benefit while minimizing side effects[68]. It has been proposed as a potential approach for patients under TB treatment failing therapy despite appropriately executed directly observed therapy (DOT)[59]. Therefore, based on TDM, the dose of isoniazid and rifampicin could be modified to optimize the therapeutic benefits and to minimize the toxicity[19]. In cases with low levels of TB-drugs concentration, an increase in the dose of TB drugs aimed at shortening the duration of treatment has been suggested[69] though few clinical studies have been performed to support it.

For practical reasons, only one or two samples should be collected post-dose. A 2-h post-dose sample approximates the peak serum drug concentration (Cmax) for most TB drugs; and adding a 6-h post-dose sample, allows the clinicians to distinguish between delayed absorption and malabsorption.

Other studies suggest that substantial variability in absorption kinetics means that the use of a single consistent time-point for TDM is unlikely to provide a reliable estimate of true TB-drug exposure[70]. Another recent study showed that individual PK variability was not able to be predicted by measurable factors, thus asserting the clinical relevance of individual drug concentration monitoring through TDM [63]. Moreover, the clinical application of TDM has been only employed in some specialized TB centres with experienced laboratories in drug concentration analysis[67]. But even in these settings with access to specialized laboratories, overall cost including serum transport and logistical coordination has limited more widespread uptake. This could be

overcome by the application of dried blood spot (DBS)[71]. Currently, TDM may be of limited practical use in poor-resource high-burden settings where it is unlikely to become available in the foreseeable future[70].

On the other hand, concentrations below the expected range for key drugs in the anti-TB regimen have been found in patients responding well to treatment[72]. So, TDM strategy requires further validation in a variety of settings and co-morbidities.

1.5 FACTORS ASSOCIATED WITH IMPAIRED PK OF TB DRUG

Antituberculosis drug pharmacokinetics could be altered by several factors, including age, sex, ethnicity, drug formulations, drug interactions, gastroenteritis or fast acetylator status for isoniazid[65,66,73,74]. Impaired absorption has been also previously suggested to occur in some patients with co-morbidities such as DM, HIV/AIDS or cystic fibrosis[75–78] .

1.5.1 DM

As it was previously mentioned DM has a negative effect on TB treatment and one of the possible underlying mechanisms could be impaired PK of anti-TB drugs. Available data are inconclusive with regard whether DM affects serum therapeutic levels of rifampicin or isoniazid. Moreover, most of the studies aimed to determine the PK of TB drugs in diabetic patients have not evaluated the relationship between plasma drug concentration and clinical outcome [77].

1.5.2 HIV

Some studies or case series have shown an association between HIV and lower concentrations of antituberculosis drug[78–81] though other researches reach conflicting results[75,82,83].

There are several reasons to support this premise. First, HIV patients might present other concomitant opportunistic infections. Second, they usually take other medications which may have other drug interactions[84,85]. Finally, HIV could itself cause malabsorption through other mechanisms such as wasting or gastrointestinal neuropathy. Therefore, unsurprisingly AIDS patients are likely to malabsorb TB drugs though this is still a controversial issue.

1.5.3 Effect of food on the PKs of TB-drugs

Several studies also suggest that bioavailability of rifampicin and isoniazid is reduced by dosing the TB drugs with meals[73,86,87] recommending to take the drug upon an empty stomach. However, other studies showed no significant difference in the time for which the serum-rifampicin remained above the minimum inhibitory concentration for *Mycobacterium tuberculosis,* suggesting that the chemotherapeutic effect is likely to be very similar[88].

Moreover, many of the anti-TB drugs can cause gastrointestinal upset (nausea, vomiting abdominal pain or loss of appetite) and patients have been reported to have stopped the medication due to adverse events[89–91]. Thus, the current official recommendations of antituberculosis treatment of the American Thoracic Society, is to provide the medication with meals if gastrointestinal intolerance persists[92]. Although antacids could relieve the adverse effects, it has also an impact on the bioavailability of the TB drugs[93]. A recent meta-analysis comparing the effect of food and antacid on the PK of first line anti-TB drugs concluded that from a PK point of view, adding antacid is a better option for patients rather than dosing with meals[87]. However, few studies have evaluated if patients dosing the TB drugs with meals are associated with treatment failure or early relapse.

1.5.4 Other factors

INTESTINAL PARASITOSIS

Intestinal parasites could vary the absorption of several drugs although to our knowledge, only one study has been performed to assess the pharmacokinetics of isoniazid drugs in patients with ascariasis[94]. It did not show up any differences in the absorption before and after treatment for ascariasis though the sample size was very small.

ACETYLATOR STAUS

Treatment with isoniazid is further complicated by a polymorphism in the expression of the enzyme system primarily responsible for its elimination, N-acetyltransferase-2 (NAT-2), resulting in trimodal elimination (slow, intermediate and fast)[95]. Distribution of acetylator phenotype varies by race and geographic region[96] and depending upon the elimination speed, the pharmacokinetics of isoniazid may be altered.

To sum up, the pharmacokinetics of rifampicin and isoniazid could be impaired in selected groups such as diabetic and HIV TB-patients. Other factors such as intestinal parasitosis or the intake of the TB drugs with food may also vary the bioavailability of these drugs.

2. THEORETICAL JUSTIFICATION AND HYPOTHESIS

There is some evidence that the metabolism of antituberculosis drugs is faulty in different risk groups of patients, such as HIV and diabetic patients. As a result, diabetic and HIV patients with TB seem to respond poorer to the treatment. Similarly, patients with intestinal parasitosis or taking the anti-TB drugs with food could be at risk of not absorbing the antituberculosis drugs and therefore could also have an impaired treatment outcome.

2.1 HYPOTHESIS ASSOCIATED WITH STUDY No. ONE

1. Serum levels of rifampicin and isoniazid are lower in diabetic patients with TB compared to non-diabetic patients with TB.
2. Serum levels of rifampicin and isoniazid are lower in HIV positive – TB patients compared to HIV negative TB patients.
3. Heavy intestinal parasitosis could interfere in the rifampicin and isoniazid PK since they might cause malabsorption.
4. In TB-HIV and DM-TB patients, impaired PKs of rifampicin and isoniazid could interfere in the treatment outcome.

2.2 HYPOTHESIS ASSOCIATED WITH STUDY No. TWO

1. To take rifampicin and isoniazid with food is associated with low levels of rifampicin and isoniazid.
2. Patients taking TB therapy with food will have a poorer response to TB treatment compared with those taking TB-drugs under fasting conditions.

3. OBJECTIVES

3.1 OVERALL AIM

The overall aim of this research is to evaluate the pharmacokinetics of rifampicin and isoniazid of patients under TB treatment and to evaluate the treatment outcome in these patients.

3.2 SPECIFIC OBJECTIVES

1. Determine the plasma levels of rifampicin in patients undergoing TB treatment either during the intensive or the maintenance phase in (See section 4.1 and 5.1.1):
    a. A group of diabetic patients and a TB control group in order to observe any differences in the absorption of the drugs in the different groups.
    b. A group of HIV patients and a TB control group in order to observe any differences in the absorption of the drugs in the different groups.
2. Determine the plasma levels of isoniazid in patients undergoing TB treatment either during the intensive or the maintenance phase in (See section 4.1 and 5.1.2):
    a. A group of diabetic patients and a TB control group in order to observe any differences in the absorption of the drugs in the different groups.
    b. A group of HIV patients and a TB control group in order to observe any differences in the absorption of the drugs in the different groups
3. To evaluate the effect of food during the drug intake in the pharmacokinetics of rifampicin and isoniazid of TB patients (See section 4.2 and 5.2):
4. To compare the treatment outcome in all subgroups of patients at the end of therapy and 6 months after having finished the treatment (4.1, 4.2, 5.1, 5.2).
5. To evaluate other possible factors affecting not only the PKs of rifampicin and isoniazid but also the outcome of TB treatment (4.1, 4.2, 5.1, 5.2):
    a. The presence of intestinal parasitosis
    b. Body Mass Index (BMI)
    c. Rifampicin/ isoniazid dosage.
    d. Treatment adherence
    e. Drug interactions
    f. Genetic factors (e.g acetylator status phenotype)
    g. Severity of TB
    h. Other factors that will be determined through the clinical history and a questionnaire (e.g. chronic diarrhoea, previous intestinal surgery).

4. METHODOLOGY

4.1 SETTING OF WORK AND DESIGN OF THE STUDIES

The study design of the first project was developed by the PhD candidate at the London School of Tropical Medicine and Hygiene when she was completing her Msc in "Tropical Medicine and International Health".

During the elaboration of her MSc Thesis, the PhD candidate collaborated with the Universidad Peruana Cayetano Heredia, a Peruvian University located in Lima. It has been the first Peruvian University in getting an international accreditation. The Faculty of Sciences and Philosophy has a related research institution called "Laboratorios de Investigación y Desarrollo" where there are different laboratories. The Laboratory in infectious diseases includes the laboratory in Mycobacterium tuberculosis.

In 2009, the PhD candidate was enrolled in this laboratory during 6 months as a medical research fellow. She coordinated the execution of the first study and subsequently designed and executed the second study, as an external researcher, under the supervision of Prof. Moore. All the studies of this research had to be approved by the ethics committee of this institution before presenting them to local ethic committees of the hospitals or health centres where the studies were carried out.

Besides the supervision of Prof. Moore, the PhD candidate was supported by a team of 7 nurses who actively participated in the recruitment of patients. Moreover, she had also the collaboration of the laboratory staff that helped in the handling and processing of blood and stool samples.

The organization of the health system in the city of Lima is quite complex. The city is divided in five extensive health administrative divisions called DISA ("Dirección de Salud"). The management of each DISA is not dependent on any other organisation since the competencies in the health area have been transferred from the ministry of health to each DISA.

The studies were carried out in the districts Villa el Salvador and San Juan de Miraflores which belong to the DISA II or "DISA Lima SUR" (see Figure 5), located in the south of Lima and it includes 13 districts (Chorrillos, Barranco, Santiago de Surco, San Juan de Miraflores, Villa el Salvador, Villa Maria del Triunfo, Lurín, Pachacamac, Punta Hermosa, Punta Negra, San Bartolo, Santa María del Mar y Pucusana).

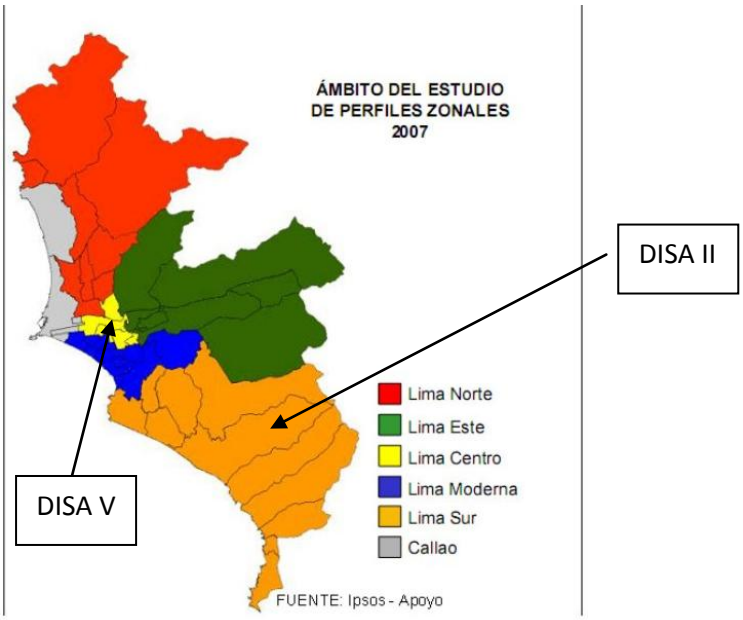


Figure 5 Health administrative divisions of Lima by DISA

The studies were also undertaken in the Hospital Nacional Dos de Mayo, a public hospital located in the centre of Lima and corresponding to the DISA number five of Lima or "DISA Lima Centro" (See figure 5). Currently, this is one the most recognized public hospitals in Lima attending a population living in one of the poorest neighbourhoods in Lima. In this context, the hospital usually attends a high number of TB cases and in particular TB with other co-morbidities such as HIV or DM.

4.2 METHODS OF STUDY No. ONE

4.2.1 Study design, participants, and setting

This cross-sectional observational study was conducted in Lima, Peru, from July to December 2009. TB patients who had received at least 15 days of treatment were recruited from health centres in Lima where directly observed therapy (6 days per week during the intensive phase and twice a week during the maintenance phase) is provided through the DOTS program of the Peruvian National Tuberculosis Program (NTP). Patients unwilling or unable to give informed consent were excluded. Patients with known HIV disease or DM were particularly sought, with the aim of recruiting at least 25 individuals for each subgroup.

4.2.2 Field methods

At the interview, a semi structured questionnaire was administered to all participants. Particular emphasis was put on detecting previous gastrointestinal surgery, ongoing chronic diarrhoea (three or more unformed stools per day for >15 days), and other factors that could contribute to malabsorption. Gender, age, height (cm), and weight (kg) were recorded, and the body mass index (BMI) was calculated.

Blood was drawn at the health centre by dedicated study staff into 10-ml lithium heparin tubes at two time points, 2 and 6 h, after directly observed TB drug ingestion, and a faecal sample was collected for parasitological analysis.

4.2.3 Laboratory methods

Blood samples were refrigerated, kept in the dark, and transported to the Universidad Peruana Cayetano Heredia (UPCH), where serum was separated (by centrifugation at 2,000 rpm for 10 min) between 2 and 5 h after sample collection. Aliquots were stored at -70°C until they were batched and transported to the pharmacokinetics laboratory of the Liverpool School of Tropical Medicine (LSTM), where rifampicin and isoniazid levels were measured by using validated high-performance liquid chromatography (HPLC) techniques.

Serum glucose levels were measured for all patients, and glycosylated haemoglobin levels (HbA1c) were measured in diabetic patients. Faecal samples were concentrated by the formol ether method and examined for the presence of ova, cysts, and parasites within 24 h of collection.

4.2.4 Rifampicin assay

All sample preparations were carried out in a darkened room. Two hundred microliters of each sample was assayed alongside a plasma calibration curve (0 to 32 µg/ml) and quality control samples with a low concentration of 1.5 µg/ml, a medium concentration of 17 µg/ml, and a high concentration of 26 µg/ml. Plasma was precipitated by using 50 µl of an ethanol-containing internal standard (butyl 4-hydroxybenzoate at 16µg/ml), followed by the addition of 1ml of methanol, and vortexed for 20 s. Tubes were incubated at 4°C for 1 h and then centrifuged at 2,000µg for 10 min. One millilitre of the upper solvent layer was transferred into a clean glass soda tube, evaporated to dryness under a stream of nitrogen at 30°C, and reconstituted in 120 µl methanol, and

100 µl of sample was then injected onto a Shimadzu LC 2010 HT HPLC system, with detection at 254 nm. Data acquisition was performed by using Chromeleon (Dionex). The compounds were separated on a Luna C8 150- by 4.6-mm 5-µm column (Phenomenex Inc.) protected by a LiChrosphere Si 60 5-µm column (VWR) using a gradient system, with the mobile phase containing solvent A (35% acetonitrile and 65% 50 mM ammonium formate [pH 5], adjusted with formic acid) and solvent B (70% acetonitrile and 30% 50mM ammonium formate [pH 5], adjusted with formic acid). The column temperature was set at 30°C. The assay was linear (r2 µ 0.99) in the concentration range of 0 to 32 µg/ml, with intra- and inter-day precisions with a <13% coefficient of variation (CV) and a <11% CV, respectively. The lower limit of quantification (LLOQ) (0.5_g/ml) has been accepted as the lowest point on the standard curve, with a relative standard deviation of less than 10% and a 5:1 signal-to-noise ratio. The determination of RIF stability following three freeze-thaw cycles showed that for all quality control (QC) samples, there was a <9% CV in rifampicin concentrations. Rifampicin stability after heat inactivation showed a <11% CV in rifampicin concentrations.

Measurements of rifampicin pharmacokinetic outcomes

Two- and six-hour plasma concentrations were determined, and for each patient, the maximum serum drug concentration (Cmax) was estimated as the higher of the two measured concentrations; the Tmax for each patient was the time point at which the Cmax occurred. Cmax values were also categorized as normal (>8 mg/litre), low (4 to 8 mg/litre), or very low (<4 mg/litre), in accordance with data reported previously [97,98].

4.2.5 Isoniazid assay

A total of 100 µl of plasma from each sample was assayed alongside a plasma calibration curve (range, 0 to 6,000 ng/ml) and quality control samples at low (40 ng/ml), medium (2,500 ng/ml), and high (5,000 ng/ml) concentrations of INH. Samples which tested above our highest level of quantification were diluted 1:4 and were reanalyzed with a fresh calibration curve and quality control samples. Each sample underwent protein precipitation with 900 _l of an internal standard (IS) (acetonitrile containing 200 ng/ml of metformin). All the IS responses for the analytical runs carried out in our samples were _15% relative standard deviation, according to the bioanalytical guidelines approved by the FDA. The samples were vortexed for 20 s and were centrifuged at 14,000 X g for 20 min; 800 µl of supernatant was transferred to clean 5-ml soda-glass tubes and evaporated to dryness under nitrogen at 30°C. Dried-

down samples were reconstituted in 100 µl of mobile phase (90% water, 10% methanol, and 0.3% formic acid) and were vortexed for 10 s. Reconstituted samples were transferred to insert vials and centrifuged at 4,700 X g for 5 min; 20 µl of the reconstitute underwent chromatographic separation on a Hypersil GOLD C18 column (150 by 4.6 mm, 3-µm particle size) (Thermo Scientific, Hemel Hempstead, United Kingdom) at 30°C using an isocratic gradient of 90% water, 10% methanol, and 0.3% formic acid at a rate of 300 µl/min. The high-performance liquid chromatography system was interfaced with a triple-quadrupole TSQ Quantum Access mass spectrometer (Thermo Scientific) with an atmospheric pressure chemical ionization (APCI) source. An E2M30 rotary vacuum pump (Edwards High Vacuum International, West Sussex, United Kingdom), an NM30LA nitrogen generator (Peak Scientific, Renfrewshire, United Kingdom), and 99% pure argon gas (10 litres) (BIP10; Air Products, Liverpool, United Kingdom) were used.

The mass spectrometer was operated in positive selective reaction monitoring (SRM) mode using transitions of m/z 138.2 to 121.1 for isoniazid and 130.2 to 60.4 for IS, an optimized collision energy of 17 eV for INH and IS, a narrow scan width (0.1 m/z) and scan time (0.1 s) for all transitions, and the data collection system operating in centroid mode. The sheath and auxiliary gas flows (nitrogen gas) were 15 and 20 lb/in2, respectively. The capillary temperature within the ion source was maintained at 250°C, the discharge current was set to 5 µA, the spray voltage was set to 4.5 kV, and the collision pressure was 1.5 mTorr (argon). All standard curves were adequately described using an equal-weighted linear regression equation for INH using the data acquisition software LCquan version 2.5.6 (Thermo Scientific, Hemel Hempstead, United Kingdom). The correlation coefficient (r2) for all INH calibration curves exceeded 0.99. The lower limit of quantification (LLOQ) (10 ng/ml for INH) was accepted as the lowest point on the standard curve, with a signal-to-noise ratio of 5:1 and a coefficient of variation (CV) of <11% for INH; the CV ranged from 2% to 11% at all other calibration levels for INH. The determination of INH stability following three freeze-thaw cycles showed that all quality control samples were within a CV of 11% for INH concentrations.

Measurements of isoniazid pharmacokinetic outcomes

For each patient, the Cmax was defined as the higher of the two concentrations measured at 2 and 6 h, and the Tmax was the time point at which the Cmax occurred. PK parameters were obtained by non-compartmental analysis using the trapezoidal rule and the linear-up-logdown method. MIC data were not available, and no additional analysis of PK-pharmacodynamic (PD) parameters was developed. Although an

internationally agreed-upon guideline for therapeutic drug monitoring is lacking, a normal isoniazid Cmax may be defined, by comparison with existing pharmacokinetic data, as 3 to 5 mg/litre after a 5-mg/kg daily dose and as 9 to 15 mg/litre after a biweekly dose of 15 mg/kg/day[73]. A Cmax level of <2 mg/litre after a 300-mg daily dose or a Cmax level of <7 mg/litre after a 900-mg biweekly dose is regarded as inadequate and is an indication for dose adjustment, according to some experts[63]. We categorized our PK data accordingly. For 5-mg/kg/day daily dosing, very low Cmax levels were <2 mg/litre, low levels were 2 to 3 mg/litre, and normal levels were >3 mg/litre; for 15-mg/kg/day biweekly dosing, very low levels were <7 mg/ litre, low levels were 7 to 9 mg/litre, and normal levels were >9 mg/litre.

4.2.6 Statistical analysis

Data were double entered into an EpiData database; checked with EpiData software, version 3.1; and analysed with STATA, version 10.

Demographic and general characteristics of the three patient groups (TB, TB-HIV, and TB-DM) were compared by using the chi-square (X^2) test for the comparison of proportions and the Student t test for continuous and normally distributed variables. Non-normally distributed data were analysed by using the Mann-Whitney test.

The primary comparison of interest was between-group serum rifampicin and isoniazid levels at 2 and 6 h post-dosing. Rifampicin pharmacokinetic data were analysed with the independent-sample t test on the natural logarithm transformed pharmacokinetic data. Patients with TB and no other co-morbidity (“TB”) were compared with (i) patients with TB and DM (“TB-DM”) and (ii) patients with TB and HIV (“TB-HIV”). A stratified analysis was performed to assess the effects of gender, age, BMI, and the dose of rifampicin administered. The dose of rifampicin and isoniazid was calculated by dividing the total milligrams of rifampicin received by the weight of patients in kg.

A multivariate analysis of variance was performed to assess the variation in rifampicin pharmacokinetics (Cmax) attributable to the presence of co-morbidity (HIV or DM), gender, BMI, and other variables that emerged from the univariate analyses.

4.2.7 Ethics and institutional review

The study protocol and consent form were approved by the ethics committee of the London School of Hygiene and Tropical Medicine (LSHTM), the institutional review

boards of the UPCH and Dirección de Salud-II (DISA-II) Lima Sur (regional Ministry of Health), and the ethics committee of the Hospital Nacional Dos de Mayo, Lima, Peru.

4.3 METHODS OF STUDY No. TWO

4.3.1 Study design, participants and setting

This observational study was conducted in Lima (Peru), from January 2012 to December 2012. People diagnosed with Pulmonary TB in DISA II district who were given the supervised treatment under DOTS programme of the Peruvian National TB programme (PNTP) were invited to participate.

The recommended scheduled by PNTP is a six days per week during the intensive phase (rifampicin, isoniazid, pyrazinamide and ethambutol) and twice a week (rifampicin and isoniazid) during the maintenance phase. The rifampicin dose is 10mg/Kg of weight and the isoniazid dose is 5 mg/kg of body weight/day during the intensive phase of treatment and during the continuation phase isoniazid is 15 mg/kg/day twice per week. Within the PNTP, TB drugs are not available as fixed-dose combinations.

Patients who were not sputum smear positive, with known HIV disease or DM, unwilling or unable to give informed consent were excluded of the study.

4.3.2 Field methods

A semi-structured questionnaire was given to all participants. Personal data, other data related to the TB disease, gender, age, height (cm) and weight (Kg) were recorded and the body mass index (BMI) was calculated. A chest radiography was performed in all participants and the scored developed by Ralph *et al*[99] was used to calculate the severity of the TB in each particular case (See methods of study 3- section 4.3). A robust and reproducible method to quantify the extent of chest radiographic abnormalities was developed.

All patients were given a diet diary where they annotated if they had eaten and the kind of food they had eaten 2 hours before, during or 1 hour after the drug intake. Sputum samples to undertake sputum smear and mycobacterium culture based on "Microscopic observation drug susceptibility" (MODS) method were collected at 30 and 60 day of treatment. At the end of therapy a conventional culture was also undertaken.

At day 30 and 60, 3 blood samples were drawn from each patient at the health centre by dedicated staff into 10ml lithium heparin tubes at 3 time points - two, four, and six hours after the directly-observed TB drug intake. One of these days, patients were required to fast at least one hour before and an hour after the drug intake.

Treatment outcome of patients was determined at end of therapy (by personal examination, chest radiography and conventional culture) and 6 months later either (by a personal interview or a phone-call).

4.3.3 Laboratory methods

All blood samples were centrifuged in the health centres (centrifugation at 2000rpm for 10 min) and aliquots of the serum was refrigerated and transported to UPCH and stored at -70ºC until batched and transported to the pharmacokinetics laboratory of the Liverpool School of Tropical Medicine (LSTM).

The pharmacokinetics of rifampicin and isoniazid were assessed by a validated High performance liquid chromatography (HPLC) method at LSTM.

4.3.4 Rifampicin and isoniazid assay

The pharmacokinetics assays for rifampicin and isoniazid have been previously described in the laboratory methods of the “Study one”.

Pharmacokinetics outcome measurement

For each patient, the Cmax was defined as the highest of the three concentrations measured at 2, 4 and 6 h, and the Tmax was the time point at which the Cmax occurred. PK parameters were obtained by non-compartmental analysis using the trapezoidal rule and the linear-up-logdown method. MIC data were not available, and no additional analysis of PK-pharmacodynamic (PD) parameters was developed. Although an internationally agreed-upon guideline for therapeutic drug monitoring is lacking, Cmax rifampicin values were also categorized as normal (>8 mg/L), low (4 - 8 mg/L) or very low (<4 mg/L) in accordance with previous works[73,97].

Normal isoniazid Cmax was defined, by comparison with existing pharmacokinetic data, as 3 to 5 mg/litre after a 5-mg/kg daily dose and as 9 to 15 mg/litre after a biweekly dose of 15 mg/kg/day[73]. Isoniazid PK data were categorized according to Pasipanodya *et al* that established that a Cmax level of <2 mg/litre after a 300-mg daily

dose or a Cmax level of <7 mg/litre after a 900-mg biweekly dose were regarded as inadequate which was an indication for dose adjustment [100].

4.3.5 Statistical analysis

The chi-square test was used for the comparison of proportions, and the Student t test or Wilcoxon rank-sum test was used for continuous variables, depending on variable distribution. The data were analysed with Stata (Stata Corp, College Station, Texas, USA) and R version 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria).

4.3.6 Ethics and institutional review

The study protocol and consent form were approved by the ethics committee of the London School of Hygiene and Tropical Medicine (LSHTM), the institutional review boards of Universidad Peruana Cayetano Heredia (UPCH) and Dirección de Salud-II (DISA-II) Lima Sur (regional Ministry of Health).

4.4 METHODS OF STUDY No. THREE

4.4.1 Rationale, study design, participants and setting

In study two, a chest radiography (CXR) was performed on all patients to assess cavitation and extent of the disease.

Recently a simple method for grading chest radiography (CXR) severity in adults diagnosed with sputum smear positive pulmonary tuberculosis (TB) was designed and validated, and shown to correlate with baseline and clinical and microbiological severity and response to treatment. A simple equation was generated to develop the CXR score as follows: proportion of total lung affected (%) + 40 if cavitation is present. This score was able to predict 2-month sputum smear status. To grade the percentage of affected lung, visual estimation of the extent of opacification, cavitation or other pathologies as a percentage of visible lung fields is made.

However, as Ralph et al acknowledge, a significant limitation of this method is the low rate of inter-observer agreement in CXR assessment which was low overall.

This difficulty (in reproducibly estimating extent of radiographic abnormality) can be overcome using novel radiologic software which is capable of accurately measuring a determined area of a radiological digitalized image, giving a precise percentage of lung

affected instead of a visual estimation. However this software is not usually available in the field and CXRs are often not performed in a suitable digital X-ray system. Using a standard digital camera, a digital picture of a conventional CXR may be obtained although this file is usually not compatible with digital X-ray software. We have developed a simple methodology based on free image editing software (GIMP, http://www.gimp.org/), which can read any type of digitalized image and provides simple capability to measure selected areas of an image. The objective of this sub-study was to evaluate the reproducibility of lung area estimation using this tool in tuberculosis patients.

We report the development and evaluation of a simple tool using free image editing software (GIMP) to accurately and reproducibly quantify the area of affected lung on the chest radiograph of tuberculosis patients.

As part of the study two, CXR were performed on all individuals recruited for the study.

All CXR films were digitalized into JPEG files by taking a photograph with conventional digital camera (See Fig 6). The digital image capture was performed by the same person, with the same camera and in the same place for all the CXRs. All CXR films were the same size and the distance from the digital camera to the films was established when the LCD monitor or the viewfinder of the camera framed the whole image. The zoom was not used in order to retain the maximum resolution of the image.

CXRs were coded and stored in a computer at the laboratory offices of Universidad Peruana Cayetano Heredia (UPCH).

4.4.2 CXR evaluation:

Two independent raters, blinded to the other's scores, evaluated the CXRs of study participants. Both were physicians specialized in Internal Medicine with more than 8 years of experience in clinical practice. For the CXR reading, they opened the JPEG files of each radiograph using the free software GNU Image Manipulation programme (GIMP 2.8). The GIMP software is available, with installation instructions, at: http://www.gimp.org/downloads/

Before commencing data collection, the two researchers involved in the study received brief training of 30 minutes about the use of the GIMP software, specifically about how to use the different commands of the software. This software permits determination of a selected area of an image by measuring the number of pixels enclosed in the selected area.

The procedure is as follows:

1. > Free selection tool command (a command that permits to select a determined area), (see Fig 1C) then use the mouse to draw a polygon around area of interest.

2. > Dockable Dialogs > histogram (the command that permits to measure this area in pixels) (see Fig 1D), a determined number of pixels is obtained.

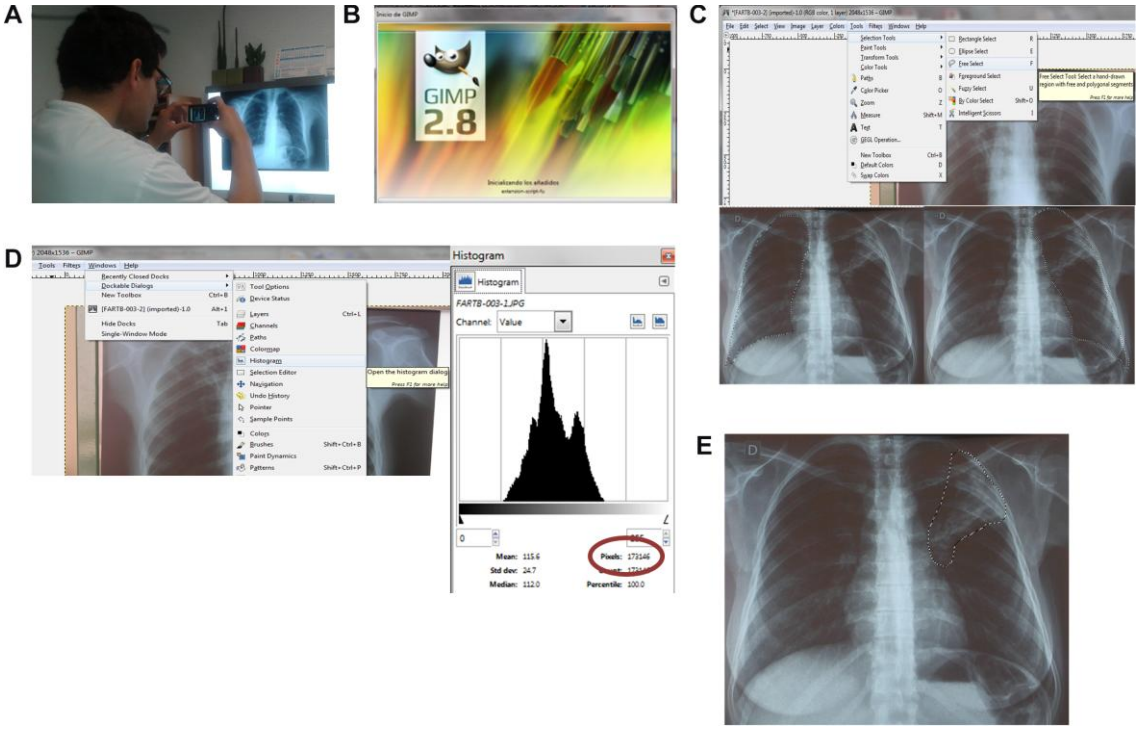


Figure 6 Flowchart of the followed methodology. A. Health worker taking a photo of a radiograph. B. Opening JPEG files with GIMP software. C. Selection of the area. D. Pixel quantification. E. Selected "affected area."

3. Enter data into a simple excel spreadsheet with built in equations that automatically calculate percentage of lung area affected and the score. Accordingly, the pixels of a selected "affected-lung "area (See Fig 1D and 1E) can be compared with the pixels of the total area of lungs (this would be the 100%) in the radiography (see Fig 1C) and the percentage of the lung affected can be calculated using a simple rule of three.

This methodology was applied to evaluate the lung affected area in each radiograph and derive a number representing the percentage of lung affected. Readers judged whether cavitation was visualized and added 40 if this was the case, to determine the final score for each radiograph, according to the method developed by Ralph et al,[9].

4.4.3 Statistical analysis

The agreement between the raters was calculated using an intraclass correlation coefficient (ICC) with a two-way mixed model and with 95% confident interval. ICC was interpreted as poor (0–0.5), moderate (0.5–0.75), good (0.75–0.9) and excellent (0.91–1) according to Portney [10]. Moreover, the variable “Proportion of lung affected” was categorized into 4 different levels of lung affectation: <25%, 25–50%, >50–75% and >75%. The “score” variable was also categorized into 4 different levels: <12.5, 12.5–25, >25–50 and >50. In both cases, the Interobserver agreement (IOA) beyond chance was evaluated by calculation of kappa coefficient. Data were analysed using STATA ver. 13.

5. RESULTS

5.1 RESULTS OF STUDY No. ONE

5.1.1 RIFAMPICIN DATA

5.1.1.1 Participant characteristics

Participant characteristics are shown in Table 1. Of 113 patients recruited into the study, PK data were available for 105 (mean age, 36.9 years; 63.8% male patients); for 5 of these patients, only one measurement was available, because either the sample was insufficient or the 6-h sampling time was missed. Sixty-one patients were in the intensive phase and 44 were in the continuation phase of their treatment; 70.5% of cases were new cases. The majority of patients (87%) had pulmonary TB; 77 had microbiologically confirmed TB, while 28 were receiving treatment based on clinic epidemiological and radiological grounds. Fifty patients had TB without a co-morbidity (TB, the baseline group for comparison), 26 had coexistent DM (TB-DM), and 29 had coexistent HIV (HIV-TB). Diabetic TB patients were significantly older and had significantly higher BMIs and significantly higher blood glucose levels than TB patients without a co-morbidity (P <0.001 for all). The median HbA1c level for diabetic patients was 8.3% (range, 4.6 to 12.8%; data were available for only 23/26 patients).

A significantly higher proportion of HIV-TB patients were male, but otherwise, this group did not differ significantly from TB patients without a co-morbidity. The mean CD4 count, available for only 14 patients, was 298 cells/μl. Eight HIV patients were receiving antiretroviral therapy, none with protease inhibitors. The dose of rifampicin (provided by IQ-farma and Infarmasa-Corporación, both from Peru) recommended by the Peruvian NTP is 10 mg/kg/day to a maximum of 600 mg per day for both the intensive and maintenance phases. The calculated dosage received was significantly lower in the DM-TB group (Table 1) and was significantly lower overall in males (9.5 mg/kg; range, 8.3 to 10.5 mg/kg) than in females (10.5 mg/kg; range, 9.3 to 11.0 mg/kg) (P : 0.004 by a Welch t test).

Table 1 Participant characteristics by subgroup

	TB n=50	DM-TB n=26	HIV-TB n=29	All n=105
Percentage male	48.0%	65.4%	**90.0% *****	63.8%
Age (years)	31.1 (18-65)	**51.3 (29-79) *****	34.1 (24-48)	36.9 (18-79)
Microbiologically confirmed TB	38 (76.0)	22 (84.6)	17 (58.6)	77 (73.3)
BMI (Kg/m²)	23.3 (18.4-29.6)	**27.5 *** (21.9-36.3)**	22.8 (15.8-34.8)	24.2 (15.8-36.3)
Diarrhoea <15 days	1 (2.0)	3 (11.5)	2 (6.9)	6 (5.7)
Chronic diarrhoea	0 (0)	0 (0)	2 (6.9)	2 (1.9)
Intestinal Surgery	4 (8.0)	4 (15.4)	2 (6.9)	10 (9.5)
Blood glucose (mg/dl) [a]	92.7 (40-159)	**178.8 (85-441) *****	92.7 (61-123)	114.2 (40-441)
Pathogenic Parasites [b,c]	4 (8.0)	1 (3.9)	4 (14.8)	9 (8.7)
Non Pathogenic parasites [b]	16 (32.0)	12 (46.2)	7 (25.9)	35 (34.0)
Intensive phase Treatment	31 (62.0)	13 (50.0)	17 (58.6)	61 (58.1)
Dose of rifampicin received in mg/kg	10.1 (7.4-12.6)	8.8 (5.7-10.9) *******	10.3 (6.7-15.8)	9.8 (5.7-15.8)

Proportions are expressed as cases/total number of patients (percent). Numerical values are expressed as means (ranges). All the tests compared the DM-TB or HIV-TB group to the TB (non-HIV, non-DM) group. Continuous variables were analyzed by using an independent *t* test, and categorical variables were analyzed with a Pearson X^2 test. BMI, body mass index. ***, *P* <0.001, compared with the TB group without a co-morbidity.
a Results were not available for one TB patient (no co-morbidity), one TB-DM patient, and two TB-HIV patients.
b Results were not available for 2 HIV patients.
c Two HIV patients had asymptomatic giardiasis, 1 patient had *Cyclospora cayetanensis*, 1 patient had *Cryptosporidium parvum*, and 1 diabetic patient also had giardiasis; in the TB group, all 4 patients had giardiasis.

5.1.1.2 Pharmacokinetic analysis

(i) Rate of rifampicin absorption (*T*max)

Unexpectedly, the *T*max was at 6 h instead of at 2 h for 61 patients (62.2%). For further analysis, subjects were therefore divided into 2 groups, (i) fast absorbers, with *T*max at 2 h, and (ii) slow absorbers, with *T*max at 6 h. Being a slow absorber (with a delayed *T*max) was not associated with gender, age group, DM or HIV co-morbidity, phase of treatment, intestinal parasitic infection, or dose received (data not shown).

(ii) Peak rifampicin level (*C*max)

Data are expressed as geometric means since the analysis was performed with the natural logarithm of the *C*max. Overall, median 2- and 6-h levels of rifampicin were 1.6 mg/litre (range, 0 to 18 mg/litre) and 3.2 mg/litre (range, 0 to 16 mg/litre), respectively.

The magnitude of the geometric mean peak rifampicin absorption (*C*max) was 4.2 mg/litre (range, 0.5 to 18 mg/litre). The geometric mean *C*max of fast absorbers was significantly higher (5.0 mg/litre) than that of slow absorbers (3.8 mg/litre) ($P = 0.05$). The rifampicin *C*max was significantly lower in male than in female patients (3.3 versus 6.3 mg/litre; $P < 0.001$), and this effect was consistent in both fast (4.4 versus 6.7 mg/litre; $P= 0.009$) and slow (2.7 versus 6.1 mg/litre; $P < 0.001$) absorbers (Fig. 7).

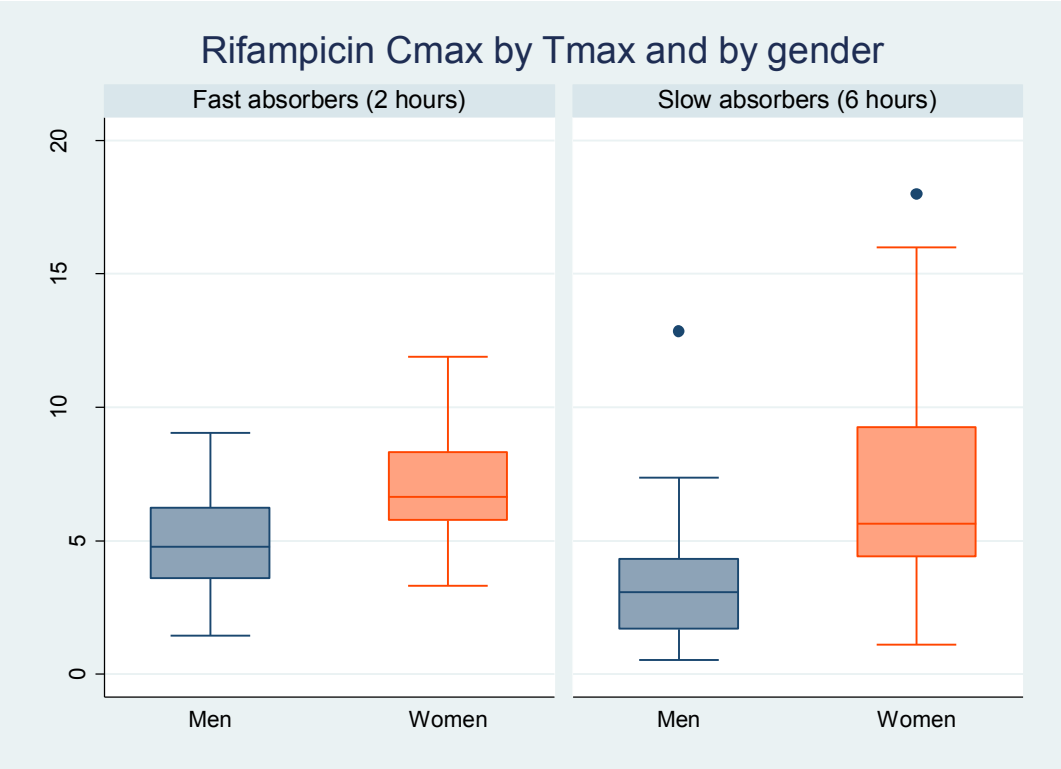


Figure 7 Rifampicin Cmax is lower in males regardless of Tmax.

Neither slow nor fast absorbers with co-morbidities had results that were significantly different *C*max from those of TB patients without co-morbidities (Table 2). The rifampicin dosage received had no discernible effect upon *C*max values among either slow or fast absorbers.

Table 2 Determinants of Cmax in fast and slow absorbers of rifampicin

	Fast absorbers (2 hours) Geometric mean Cmax mg/L, n=37	p value	Slow absorbers (6 hours) Geometric mean Cmax mg/L, n=61	p value
TB-comorbidity				
DM-TB	5.1	0.99	3.4	0.29
HIV-TB	4.8	0.76	3.1	0.10
TB	5.1	----	4.5	----
Sex				
Male	4.4	0.009	2.7	<0.001
Female	6.7	----	6.1	----
Phase of Treatment				
Intensive phase	5.5	0.64	3.4	0.06
Maintenance phase	4.9	----	4.7	----
Age group				
18-30 years	5.0	0.93	4.4	0.61
31-40	4.9	----	3.5	----
>40 years	5.2	----	3.5	----
Intestinal parasites				
Yes	3.9	0.40	3.0	0.49
No	5.1	----	3.8	----
Overall Geometric mean	5.0		3.8	

Notes: Numerical values are expressed in: geometric mean; The TB-co-morbidity group compare DM-TB or HIV-TB groups to TB (non-HIV, non-DM) group. All the variables were analyzed using the independent sample t-test on the natural logarithm transformed data.

Thirty-six patients (34.3%) had undetectable rifampicin levels at 2 h, two of whom also had undetectable levels at 6 h. In the fast-absorber group, 10 patients (27.0%) had *C*max values of <4 mg/litre, 23 (62.2%) had rifampicin levels between 4 and 8 mg/litre, and only 4 (10.8%) had values that are regarded as acceptable normal levels. For slow absorbers, 31 (50.8%) had *C*max values of <4 mg/litre, 20 (32.8%) had levels between 4 and 8 mg/litre, and 10 patients (16.3%) had normal levels (>8 mg/litre) (Fig. 8).

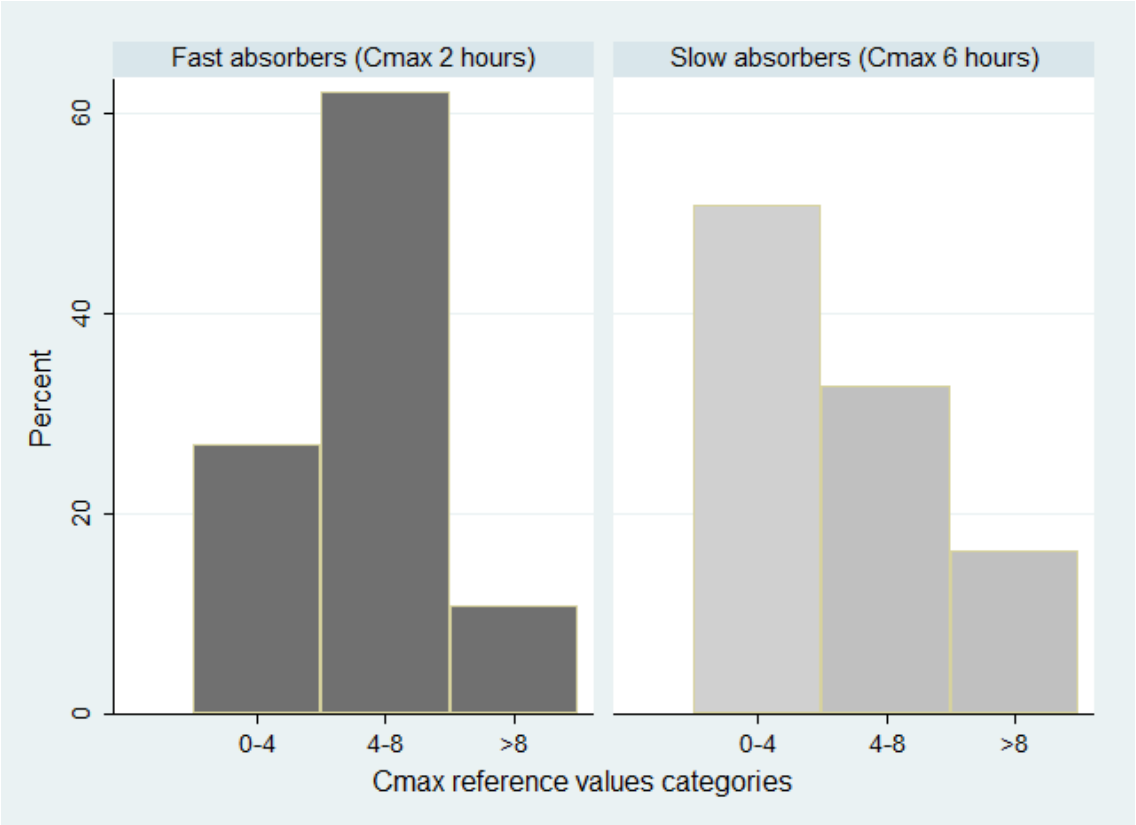


Figure 8 Frequency distribution of Cmax categories by slow- and fast- absorber subgroups

A multivariate analysis was performed, where the dependent variable was "the natural logarithm of *C*max." We built a model to assess the independent effect of the time of absorption, gender, co-morbidity (DM or HIV associated with TB), and rifampicin dose received. The rifampicin dose received was not found to have an effect upon the *C*max. However, males were found to receive lower doses than females. Therefore, it was decided to include the variable rifampicin dose received in the multivariate analysis. Other variables, such as age, serum glucose level, or the presence of intestinal parasites, were not significantly associated and were excluded during the building of the model (Table 3). The rifampicin dose received did not significantly influence the *C*max in our model. However, males were found to have *C*max levels 1.9 times lower than those of females (confidence interval [CI], 1.42 to 2.56; *P* value, <0.001), and fast absorbers also differed significantly compared to slow absorbers (0.71; CI, 0.54 to 0.93; *P* value, 0.012) in our model.

Table 3 Multivariate regression model of independent association of various variables with exposure to rifampicin

		Proportional Difference	CI	p-value
Tmax (6 hours)		0.71*	0.55-0.93	**0.012**
Sex (female)		1.91†	1.42-2.56	**<0.001**
Rifampicin dose received		1.05	0.96-1.14	0.299
Co-morb.	**HIV**	0.98	0.71-1-37	0.926
	DM	0.99	0.71-1.37	0.931

Note: The model was considered based on the natural logarithm of the Cmax values. The proportional difference was calculated as the exponential of the coefficient obtained for each variable in the multivariate model. Interpretation of the proportional difference: * Levels of Cmax in slow absorbers were 29% lower than levels of Cmax in fast absorbers. † Levels of Cmax in women were 91% higher than in men.

Since (unexpectedly) many patients had undetectable levels at 2 h and many others had rifampicin levels that were higher at 6 h than at 2 h, the area under the curve could not be calculated.

It is recommended that rifampicin must be taken on an empty stomach, as a modest reduction in absorption was previously observed when rifampicin was taken with food. Information on oral intake was available for only 48 patients, 12 of whom fasted for at least 2 h before taking the treatment. In this small, underpowered subgroup analysis, oral intake was not associated with a delayed *T*max or with a reduced *C*max. Two Peruvian companies provide rifampicin to the NTP. Health centres do not necessarily receive only a single brand, and it was possible to reliably identify the manufacturer for only 48 patients. Thirty-eight patients (80.9%) had been treated with drugs from manufacturer “A,” while 9 (19.2%) had been treated with drugs produced by manufacturer “B.” The median plasma rifampicin *C*max values achieved did not differ between manufacturers (4.5 mg/litre for manufacturer A [range, 0 to 15.6 mg/litre] versus 4.9 mg/litre [range, 1.5 to 12.9 mg/litre] for manufacturer B; *P* =0.7).

The treatment responses of 99 out of the 105 TB patients were evaluated 6 months after the completion of treatment. Ten patients were found to have multidrug-resistant TB (MDR-TB) and changed treatment schema after the PK analysis and were therefore not included in this subanalysis. A further 29 patients either had abandoned treatment and restarted several months later or were lost to follow-up. Of the remaining evaluable 60 patients, 55 were considered completely cured and relapse free 6 months after finishing treatment (20 of them without a microbiologically confirmed diagnosis), and 5 had an unfavourable outcome. Out of these 5 patients, 2 of them were diabetic

patients, 2 were HIV positive (1 of the HIV-positive patients had extrapulmonary cerebral TB without improvement despite prolonged therapy), and 1 patient had no co-morbidity. Four of these 5 patients had a rifampicin absorption delay (*T*max at 6 h instead of at 2 h), 3 of them had very low rifampicin Cmax levels, and the other 2 had low levels (4 to 8 mg/litre).

Among the 55 patients with a good outcome, 31 patients (56%) had delayed absorption, 18 patients (33%) had rifampicin Cmax levels of <4 mg/litre, 26 (47%) had low levels, and 8 (15%) had normal *C*max levels. For 3 of these patients with a good outcome, neither the *T*max nor the *C*max could be calculated because only 1 blood sample was available.

5.1.2 ISONIAZID DATA

5.1.2.1 Participant characteristics

The general characteristics of the patients are described in Table 4. PK data were not available for 6 out of 113 patients initially recruited due to inefficient processing of the sample or difficulties on venipuncture. In 4 of the remaining 107 patients, the 6 hour sampling time was missed. 62 patients (57.9%) were sampled whilst receiving treatment in the intensive phase and 45 in the continuation phase.

As previously reported for this population (27), patients with DM were significantly older and had a significantly higher body mass index (BMI) than TB patients without DM ($p<0.001$ for both).

Table 4 Participant characteristics by subgroup

	TB n=52	DM-TB n=25	p-value	HIV-TB n=30	p-value	Total n=107
Sex (Male)	26 (50%)	16 (64%)	0.2	26 (86.7%)	**0.001**	67 (63.81)
Age (years)	29 (22.5-36)	50 (44-58)	**<0.001**	35 (30-38)	0.3	35 (26-44)
Confirmed TB[1]	40 (76.9%)	21 (84%)	0.5	17 (56.7%)	0.055	78 (72.9)
BMI (Kg/m²)	22.9 (21.23-24.89)	27 (24.9-29.8)	**<0.001**	22 (20.8-24.6)	0.5	23.7 (21.5-25.8)
Chronic diarrhoea[2]	0 (0%)	0 (0%)	---	2 (6.7%)	0.059	2 (1.9)
Intestinal Surgery[3]	4 (7.7%)	4 (16%)	0.3	2 (6.7%)	0.9	10 (9.35)
Blood glucose (mg/dl)	95[4] (80-102)	119.5[1] (111-211.5)	**<0.001**	92.5[4] (81-102.5)	0.8	98[1] (84-114)
Pathogenic Parasites	4 (7.7%)	1 (4%)	0.5	4[5] (14.3%)	0.3	9[2] (8.6)
Non-pathogenic parasites	16 (30.8%)	12 (48%)	0.1	8[5] (28.6%)	0.8	36[2] (34.3)
Intensive phase Treatment	33 (63.5%)	13 (52%)	0.2	17 (56.7%)	0.5	62 (57.9)

Notes: Proportions are expressed in: cases /total N (%); Numerical values are expressed in: median (Inter quartile range); BMI: Body mass Index. All the tests compare DM-TB or HIV-TB groups to TB (non-HIV, non-DM) group; Continues variables were analyzed using independent t-test and categorical variables were analyzed with Pearson X^2 test. 1: Confirmed TB are microbiologically confirmed TB cases; 2: Chronic diarrhea was defined as persistence of liquid depositions for more than 15 days; 3: Intestinal surgery was surgery related to gastro-intestinal tube; 4: In two HIV patients one DM patient and two TB control patient, the blood glucose could not be tested.5: In 2 HIV patients, faeces samples were not processed.

Overall mean dose received during the intensive daily dosing phase was 5.15mg/Kg/day though because of higher BMI, patients with DM received a lower daily dose of isoniazid compared with the TB only group (4.47 vs. 5.3 mg/Kg, p=0.02, t-test) (figure 1). During the continuation phase, the mean biweekly dose received on treatment days was 12.8 mg/Kg/day, significantly below the recommended dose of 15mg/Kg/day (p<0.001) and with 89% of patients receiving lower than the nominal dosage. This effect was particularly marked in the TB-DM group (11.6 vs. 13.3 mg/Kg/day, p=0.015) (figure 1). Mean maintenance phase dose of isoniazid received was lower in males than females (12.22 vs. 13.48 mg/Kg/day, p= 0.008).

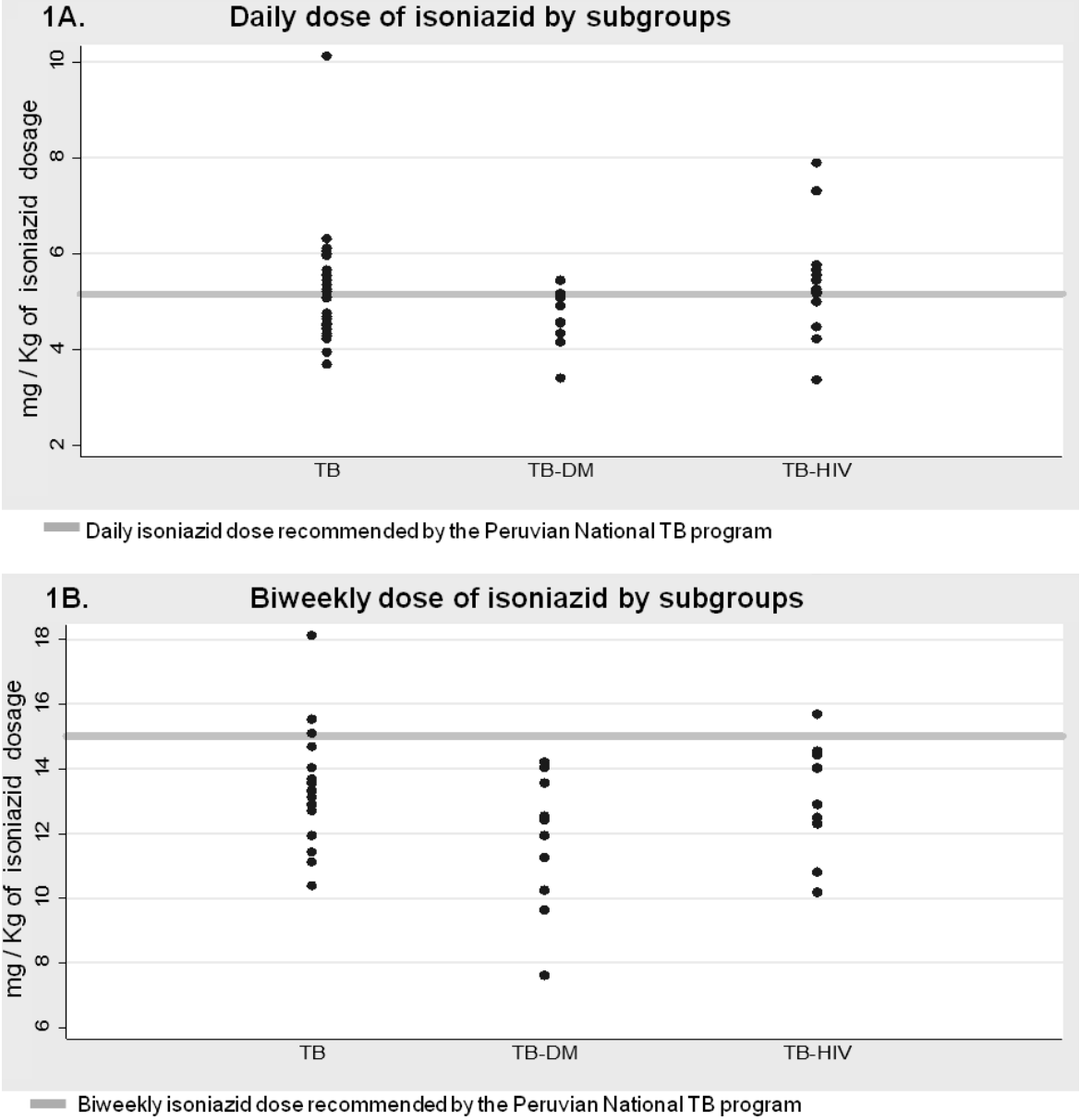


Figure 9 Daily (1A) and biweekly (1B) isoniazid dose, as recommended by the Peruvian National TB program.

5.1.2.2 Pharmacokinetic analysis

T_{max} occurred at 2 hours in 86.4% of intensive phase patients and 90.7% of continuation phase patients. In intensive phase patients median isoniazid concentration was 2.55 (IQR 1.67-3.5) mg/L at 2 hours and 0.59 (0.3–2.31) mg/L at 6 hours. Median C_{max} was 2.77mg/L (1.75-4.67). In continuation phase patients, median concentrations of isoniazid at 2 and 6 hours were 8.71 (4.01 -23.27) mg/L and 3.1 (1-5.81) mg/L respectively. The median C_{max} was 8.74 (4.21-23.03)mg/L. The percentage of patients

in the intensive phase group who did not reach a reference value at 2 hours after drug intake of at least 2 mg/L (very low levels) was 34%; 33.3% of patients taking the isoniazid biweekly did not achieve a reference value of 7mg/L at 2 hours. Median AUC_{0-6} was 9.71 (5.87 -13.31) mg*h/L for intensive phase patients and 37.8 (19.2 – 82.26) mg*h/L for continuation phase patients. Since information about NAT2 genotype of participants was not available, a bivariate normal mixture model was fitted to the distribution of the half-lives to estimate the proportion of acetylator phenotypes from the data. Mixtures of normal distributions were fitted to PK parameter distributions using maximum likelihood in R. The best model for the distribution was bimodal in which 53% of the population was estimated to be fast/intermediate acetylators with a mean half life of 1.48h and 47% slow or intermediate acetylators with a mean half life of 5.25h.

Univariate analysis of factors influencing PK parameters is presented in Table 5. The relationship between weight-adjusted dose and PK exposure / AUC_{0-6} was demonstrated to be linear across the wide range of doses studied (figure 10).

Table 5 Determinants of INH Cmax and AUC_{0-6} in daily and twice weekly dose

	Daily dose of INH						Twice a week INH dose					
	n	median Cmax (mg/L)	p-value	n*	AUC_{0-6} (mg·h/L)	p value	n	median Cmax (mg/L)	p-value	n*	AUC_{0-6} (mg·h/L)	p value
TB-comorbidity												
DM-TB	11	2.6	1	11	10.39	0.6	13	4.4	0.3	11	54.26	0.2
HIV-TB	16	2.6	0.8	13	9.72	0.6	13	9.9	1	12	53.64	0.6
TB	32	3	----	27	11.05	----	18	8.6	----	16	62.45	----
Sex												
Male	36	2.8	1	31	10.23	0.9	29	8.6	0.2	26	46.28	0.2
Female	23	2.8	----	20	11.1	----	15	21.4	----	13	79.71	----
Fasting with therapy												
Fasting group	6	3.1	0.4	6	11.06	0.2	6	16.6	1	4	71.52	1
Non fasting group	25	2.1	----	20	6.96	----	9	7.7	----	8	53.17	----
Age group												
18-30 years	22	3	0.8	20	10.61	0.6	17	9.9	0.9	15	40.51	0.7
31-40	23	2.5	----	18	8.77	----	12	7.4	----	10	31.45	----
>40 years	14	2.9	----	13	9.71	----	15	8.8	----	14	28.32	----
Intestinal parasites												
Yes	4	2.1	0.6	3	4	0.3	5	9	0.9	5	34.81	0.9
No	53	3	----	46	10.12	----	39	8.6	----	34	38.65	----
Bacteriologically confirmed TB												
Yes	44	2.8	0.6	36	9.6	1	31	9.9	0.9	26	38.9	0.6
No	15	2.5	----	15	9.7	----	13	8.6	----	13	19.6	----
Overall median	59	2.8	----	51	9.71	----	44	8.7	----	39	37.81	----

Notes: Numerical values are expressed in: median; The TB-co-morbidity group compare DM-TB or HIV-TB groups to TB (non-HIV, non-DM) group. All the variables were analysed using the non-parametric Wilcoxon test and the Kruskal Wallis test for the age group variable. *: n related to Cmax is slightly different to n related to AUC_{0-6} because AUC_{0-6} could not be calculated in case of undetectable levels or delayed absorption (Cmax at 6 hours).

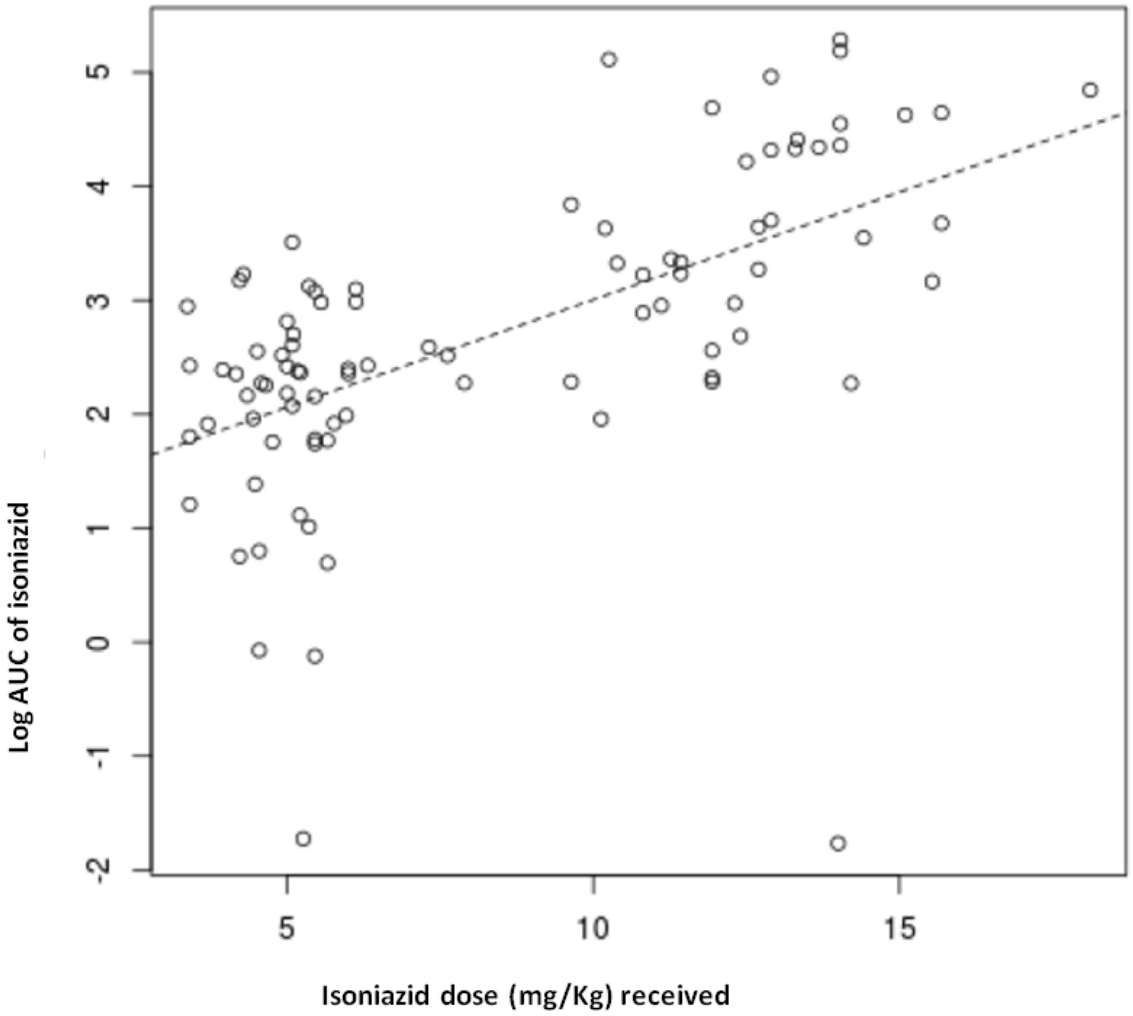


Figure 10 Relationship between dosage and exposure of isoniazid

Information on food intake of patients was only available for 48 patients, 12 of whom fasted for at least 2 hours before taking the drugs as recommended. Although both C_{max} and AUC_{0-6} appeared reduced in non-fasting patients this was not a statistically significant finding (table 5). Notably, none of the six patients in the intensive phase group who fasted before drug intake had 2-hour concentration levels of <2 mg/L compared to 12/25 (48%) of intensive phase patients who did not fast p= 0.04, Fisher' exact test) though this was not true in the maintenance phase group (33% fasting vs. 22.2% non-fasting had 2 hour-concentration levels below 7mg/L; p=0.4). In univariate analysis, C_{max} and AUC_{0-6} were unaffected by age, gender, DM or HIV, in either intensive or continuation phases (Table 5 and Figure 11).

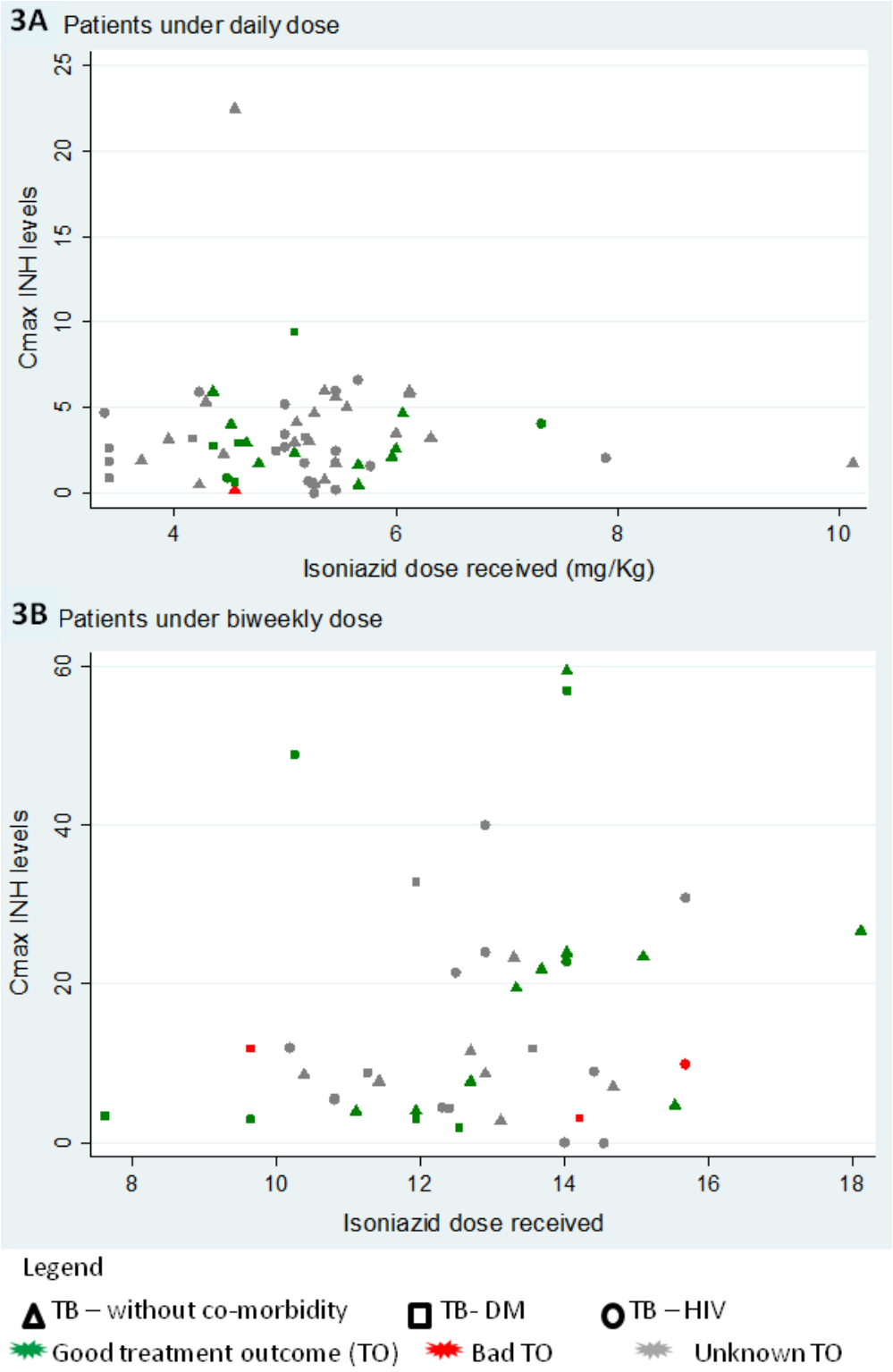


Figure 11 INH Cmax levels by co-morbidity and treatment outcome for patients who received a daily (3A) or biweekly (3B) dose

Multivariate models with log-transformed PK parameters as dependent variable were used to further evaluate the effect of the co-morbidities of interest, while taking into

account the effect of dose. For both AUC_{0-6} and $C_{max,}$ the isoniazid dose received was the most significant covariate (p= 0.028 and p=0.029 respectively) whereas neither co-morbidity (having HIV infection or DM), age, sex, fasting or intestinal parasitosis significantly influenced the PK parameters (data not shown).

MIC data were unavailable and therefore PK-PD parameters could not be estimated.

5.1.3 TREATMENT OUTCOME

Treatment outcome was available six months after completion of therapy in 99 of 107 patients. After exclusion of ten patients diagnosed with multidrug resistant TB, 26 who either abandoned treatment or did not properly complete treatment and 22 others lacking a microbiologically confirmed diagnosis, 41 patients were included in the treatment outcome sub-analysis. 37 patients were regarded as cured and four had an unfavourable outcome - two patients (one HIV+) died on treatment, one patient had a drug-susceptible relapse within 6 months after apparent cure and in one patient treatment was prolonged for persistence of TB despite treatment.

In both intensive and maintenance phase sampled groups, there was no significant difference in the C_{max} of patients with a good treatment outcome compared to patients who had treatment failure or relapse Three of the four patients with an unfavourable outcome had a very low Cmax when sampled either in the intensive or continuation phase compared to 14/34 (41.2%) subjects with very low levels in those with favourable outcomes.

5.2 RESULTS OF STUDY No. TWO

5.2.1 GENERAL DATA

5.2.1.1 Participant characteristics

Sixty patients were initially included in the study. The general characteristics of the patients are summarized in table 6. Patient had a median age of 32.7 (IQR 23.7-45 years) and 34 of them (56.7%) were male. The median body mass index (BMI) was 21.8 (19.8-24.4). Only 3 (3.33%) and 4 (7.7%) presented chronic diarrhoea and intestinal parasite respectively and 4 of them required hospitalization. Most cases (88.3%) were new TB cases whereas 7 of them (11.67%) were a late relapse of a previous TB episode.

Table 6 General characteristics of the patients

	TB n (%)
Sex (Male)	34 (56.7)
Age (years)	32.7 (23.7-45)
BMI (Kg/m²)[a]	21.8 (19.8-24.4)
Chronic diarrhoea	2 (3.33)
Intestinal parasite	4 (7.7)
Hospitalization	4 (6.67)
HIV (n=56)	2 (3.57)
TB type	
New	53 (88.3)
Relapse />6 months)	7 (11.67)
Sputum smear +	28 (46.7)
++	17 (28.3)
+++	15 (25)
Symptoms	
Weight loss (n=59)	37 (62.71)
Cough	55 (91.67)
Fever (n=59)	34 (56.67)
Thoracic pain	40 (66.67)
Dyspnoea	28 (46.67)
Haemoptysis	21 (35)
Sweating	37 (61.67)
Anorexia	28 (46.47)
TB diagnosis	
Conventional culture	44 positive (not undertaken in 16)
MODS	41 pos, 7 neg
R dosage (mg/Kg/day)	10.2 (9.6-11.2)
H dosage (mg/Kg/day)	5.1 (4.8-5.6)
E dosage ((mg/Kg/day)	20.3 (19.1-22.4)
P dosage (mg/Kg/day)	25.4 (23.9-28)
Radiograph (Ralph score)*	22.6 (12.6-40.2)

a. Numbers are expressed in median and Interquartile range. *Ralph score obtained in the Chest radiography evaluation = % of affected lung + 40 (if cavitation is present)

The median rifampicin, isoniazid, ethambutol and pyrazinamide dosages received were consistent with the dosages recommended by the PNTB.

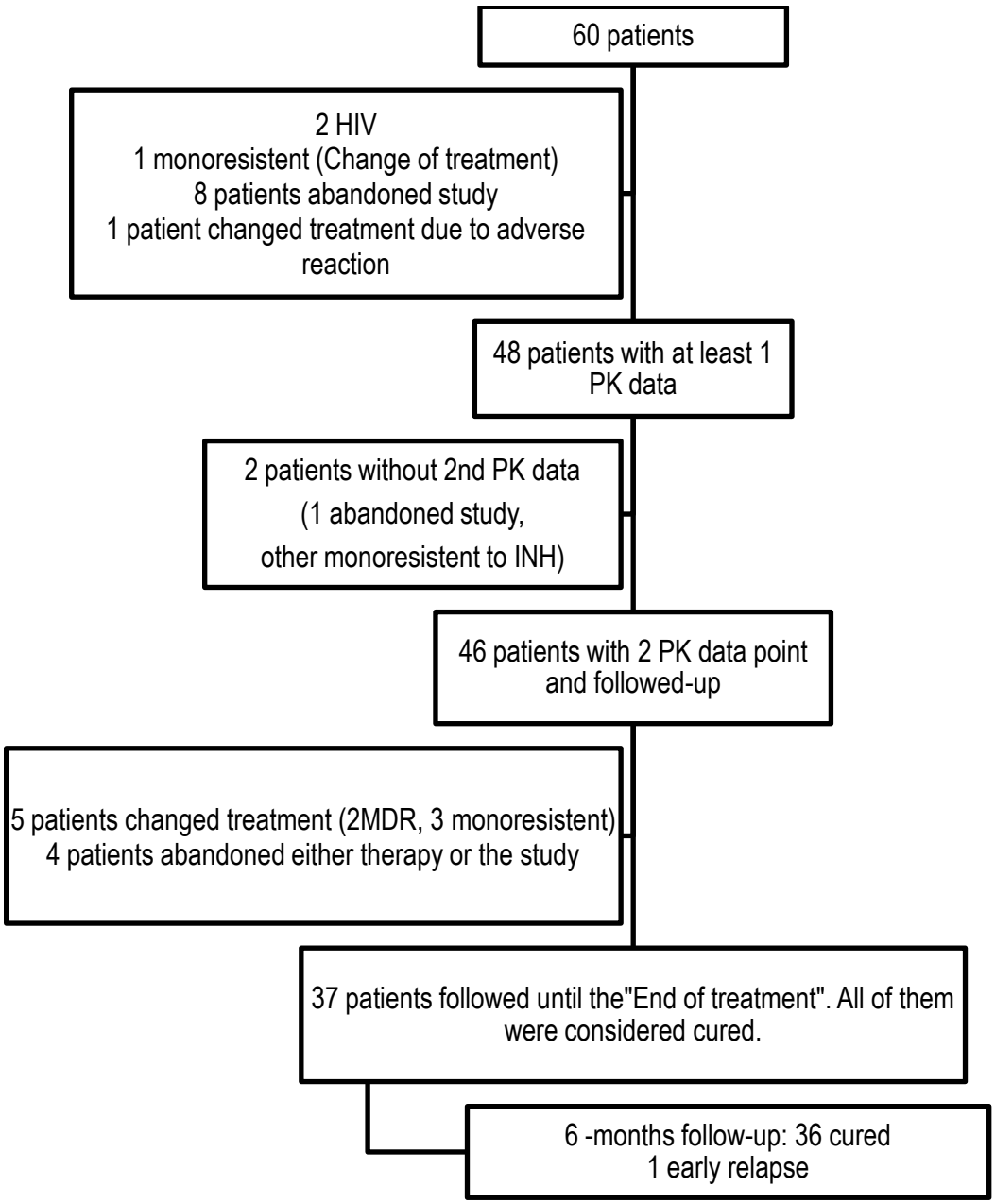


Figure 12 TB diagram about patients recruitment and follow-up during the study.

Eight patients abandoned the study in the first days before the first blood sample. Two patients were diagnosed with HIV after their inclusion in the study and they were subsequently withdrawn of the study. Two patients changed the treatment schedule before the first blood sample was carried out (one because of an adverse reaction and one because of mono-resistance to isoniazid). Forty eight patients (mean age 36.4

years; 58.3% male) had at least one PK data point and they were enrolled in the PK analysis. Six of them were on their second episode of TB (See figure 12).

However, 12 patients could not be further followed-up for treatment outcome (seven patients changed the therapy because of drug resistance and five abandoned either the therapy or the study) and 37 participants were included in the follow-up evaluation.

5.2.1.2 Diet diaries

The information on the dietaries was collected in the 37 patients that were followed up. During the intensive phase, patients properly fasted (only drinking water) on a median of 2% of the treatment days (IQR 1-7.5) and they took the TB drugs with water or any other drink (juice, cereal or carbonated beverages) without solid food a median of 4.5% of the treatment days (interquartile range 1-11.5). The rest of the treatment days, patients consumed some food during drug intake.

During the maintenance phase, patients fasted (only drinking water) with the drug intake a median of 1% of the treatment days (0-3) and they took the TB drugs either with water or with a drink-without-meal a median of 4% of the treatment days (0-10). The rest of the treatment days, patients consumed some food during drug intake.

In table 7, there is a more detailed summary of the diet diaries of the participants of the study regarding the moment in which they ingested the food (before, during or after the drug intake) and also about the kind of food they consumed.

Table 7 Information of dietaries about the ingest of food before, during and after drug intake

	Intensive phase			Maintenance phase		
Up to 1 hour before of drug intake	Nº days fasting		6 (1.5-20)	Nº days fasting		4 (1-12)
	Nº days eating		36 (25-47)	Nº days eating		9 (4-18)
	% fasting		18.3 (3.1-41.7)	% fasting		38.7(11.1-62.5)
	% of treatment days that consumed	carbohydrate	77%	% of treatment days that consumed	CH	57%
		Fat	3%		Fat	10%
		Protein	46%		Protein	33%
		fruit	12%		Fruit	6.9%
During drug intake	Nº days fasting		11.5 (3-27.5)	Nº days fasting		3 (1-14)
	Nº days eating		34.5 (10-43)	Nº days eating		9 (3-21)
	% fasting		24.2 (7.7-73.4)	% fasting		18.8 (6.4-79.3)
	% of treatment days that consumed	Carbohydrate	51.7%	% of treatment days that consumed	CH	37.5%
		Fat	0%		Fat	0 %
		Protein	2%		Protein	0 %
		Fruit	2.2%		Fruit	0 %
Up to one hour after drug intake	Nº days fasting		38 (23.5-43.5)	Nº days fasting		15 (9-22)
	Nº days eating		10 (3.5-20)	Nº days eating		2 (0-5)
	% fasting		79 (52.2-92.7)	% fasting		91.3 (71-100)
	% of treatment days that consumed	CH	6.9%	% of treatment days that consumed	CH	4.3%
		Fat	0%		Fat	0 %
		Protein	6.9%		Protein	3.1%
		fruit	11.3%		Fruit	0%
All the numbers are expressed in Median and IQR. The percentages have been calculated as the median of the percentage of each variable.						

5.2.1.3 Follow-up

All 37 patients evaluated at the end of therapy were considered cured. Thirty seven patients were evaluated at six months after the end of the therapy and 36 were considered cured and only one relapsed two months after having finished the therapy. This 47 year-old male patient correctly completed the first line TB therapy with clinical and radiological improvement although a fibrotic scar could be appreciated in the apex lobe of the right lung in the final chest radiograph. TB culture was negative at the end of therapy. Two months later he restarted TB therapy because an early relapse was diagnosed. The Rx-score of this patient had been 42 (median Ralph score 22.6). He had fasted 16% of the time during the intensive phase and had not fasted on any occasion in the maintenance phase.

Neither the Rx score nor consumption of food with drug intake or pharmacokinetics of rifampicin or isoniazid were associated with a poorer treatment outcome (data not shown).

5.2.2 PHARMACOKINETICS RESULTS

5.2.2.1 Pharmacokinetics of rifampicin

Did rifampicin exposure at each time-point differ between fasted and non-fasted treatment days?

Overall, median serum rifampicin levels at two, four and six hours were 3.25, 6.08 and 4.2 mg/L respectively during the non-fasting day and 6.49, 6.08 and 4.23 mg/L during the fasting day (See figure 14).

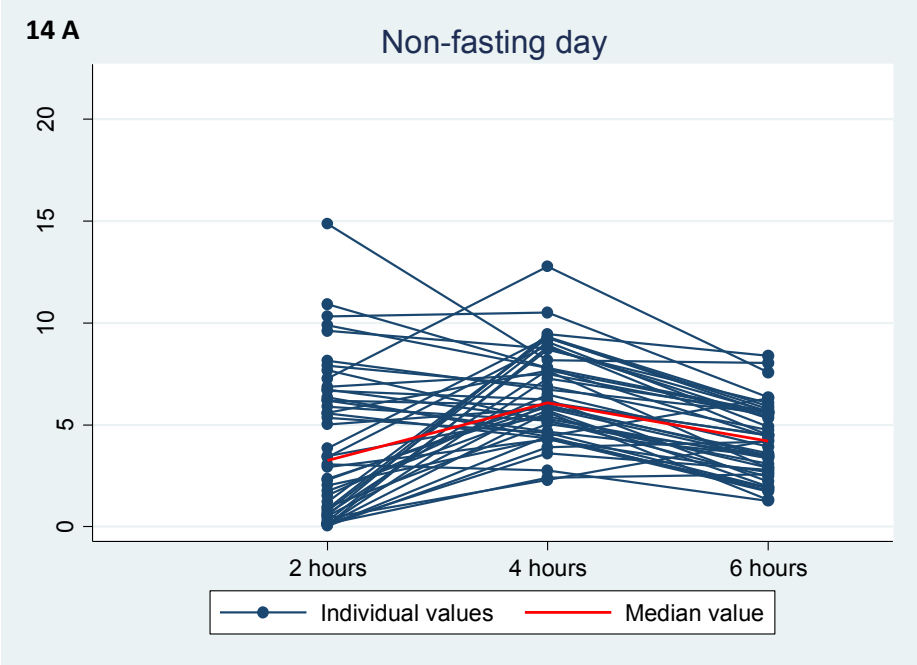
Did rifampicin Cmax or AUC_{0-6} differ between fasted or non-fasted treatment days?

It was observed a weekly difference in the Cmax at the non-fasting day (6.59 mg/L) compared with the Cmax at the fasting blood-sampling day (7.02 mg/L) (p: 0.054) and the AUC_{0-6} in the non-fasting blood sampling day was 24.31 mg·h/l vs. 28.64 mg·h/l during the fasting day (p: 0.002).

Did effect of fasting on rifampicin Cmax or AUC_{0-6} differ between males and females?

The rifampicin *C*max was significantly lower in male compared with female patients (6 versus 8.3 mg/litre; p: 0.035), in the non-fasting day but this effect was not consistent when the blood test was taken in the fasting day (6.73 versus 7.55 mg/litre; p: 0.09).

Similarly, the AUC in male was lower in male compared with female patients (22 *vs*. 27.27 mg·h/l) in the non fasting blood-sampling and in the fasting day (27.85 *vs*. 31.75 mg·h/l) although the differences were not statistically significant; (p:0.08 and p:0.09 respectively).



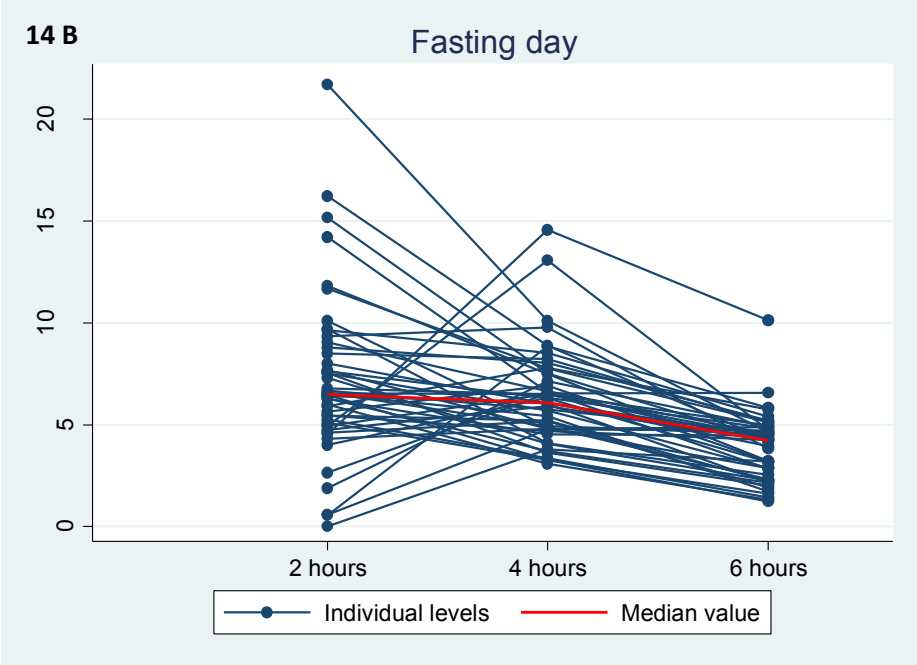


Figure 14 Pharmacokinetic levels of rifampicin in the non-fasting (14A) and fasting (14B) day

Effect of fasting on Tmax

On fasting days, Tmax occurred at 2, 4 or 6 hours in 68.8%, 27.1% and 4.2% respectively. On non-fasting days, Tmax occurred at 2, 4 or 6 hours in 34.8%, 56.5%, and 8.7% respectively. Moreover, there were significantly differences in the Tmax in the non-fasting day compared with the fasting day. (p: 0.005).

Tmax was not associated with gender, age group, intestinal parasitic infection, or dose received (data not shown) in both non-fasting and fasting day.

Categorization of Cmax: Therapeutic *vs.* non therapeutic levels.

When non-fasting, three patients (6.52%) had *C*max values of <4 mg/L, 28 (60.9%) had rifampicin levels between 4 and 8 mg/L, and 15 (32.6%) had values that are regarded as acceptable normal levels (>8mg/L). When the blood sample was taken during the fasting day, 1 (2.1%) had *C*max values of <4 mg/L, 30 (62.5%) had levels between 4 and 8 mg/L, and 17 patients (35.4%) had normal levels (>8 mg/L) (Fig. 15).

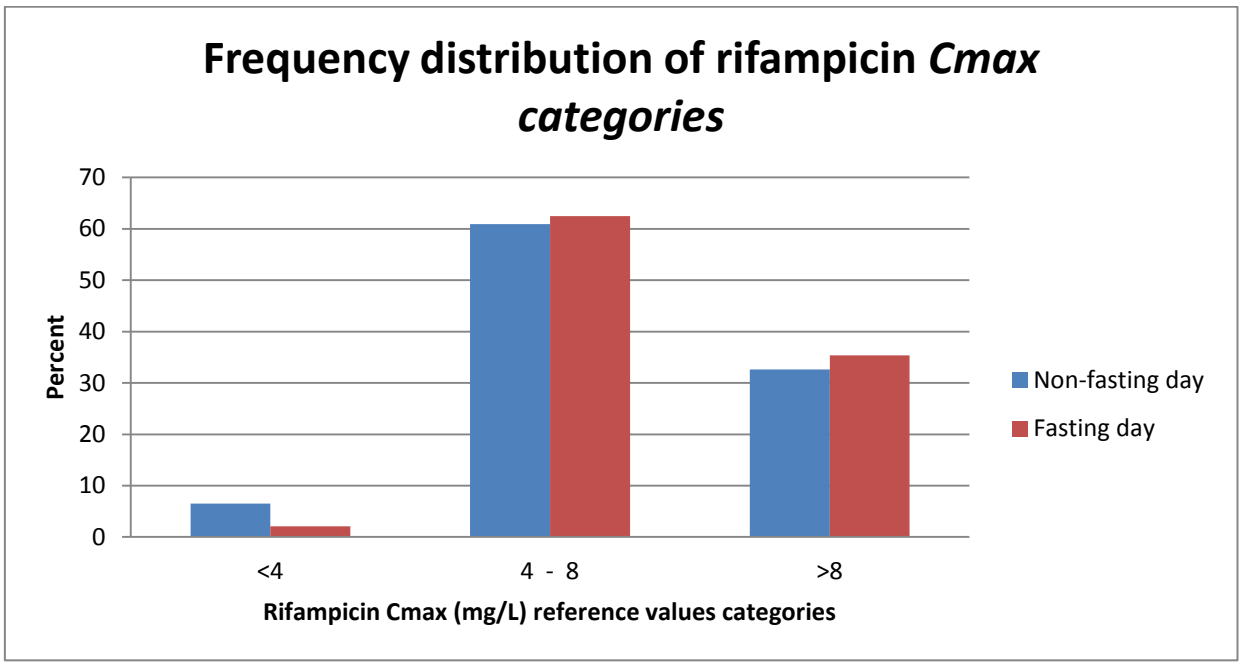


Figure 15 Frequency distribution of Cmax categories during the non-fasting and the fasting day.

Interestingly, there was a correlation between the Cmax of rifampicin when the drug was taken with food and rifampicin dosage received (coefficient Spearman 0.37, p: 0.011) whereas this correlation was not observed when the sample was a fasting-blood sample (coefficient Spearman 0.15, p: 0.31).

Multivariate analysis

1. A multivariate analysis was performed through a multilevel lineal model, where the dependent variable was the logarithm of the Cmax rifampicin. We built a model to assess the independent effect of fasting during the drug intake, the rifampicin Tmax and the gender on the rifampicin Cmax.

In this model, rifampicin Cmax in the fasting day was found to be 15% higher than rifampicin Cmax during the non-fasting day (Confident Interval: 1.01 – 1.3, p<0.036). Females had 20% levels higher than males (p: 0.027). The effect of Tmax did not influence the Cmax (Tmax 4h: 0.98, p: 0.901; Tmax 6h: 1.11, p:0.676).

Table 8 Multilevel linear model of the independent association of variables with rifampicin exposure

Variable	Proportional difference	CI	p-value
Fasting	1.15*	1.01-1.3	**0.036**
Tmax 4h	0.98	0.77-1.26	0.901
Tmax 6h	1.11	0.69-1.78	0.676
Sex	1.2†	1.02- 1.41	**0.027**
Note: The model was considered based on the natural logarithm of the Cmax values. The proportional difference was calculated as the exponential of the coefficient obtained for each variable in the multilevel linear model. Interpretation of the proportional difference: * Rifampicin Cmax in the fasting day was 15% higher than rifampicin Cmax during the non-fasting day. † rifampicin Cmax in female were 20% higher than rifampicin Cmax in males.			

2. A multivariate analysis was performed through a multilevel lineal model, where the dependent variable was the logarithm of the rifampicin AUC_{0-6}. We built a model to assess the independent effect of fasting during the drug intake, the rifampicin Tmax and the gender on the rifampicin AUC_{0-6}.

In this model, rifampicin AUC_{0-6} in the fasting day was found to be 14% higher than rifampicin AUC_{0-6} during the non-fasting day (Confident Interval: 1.01 – 1.28, p<0.021). Females had 2% levels higher than males (p: 0.027). When Tmax occurred at 4h, rifampicin AUC_{0-6} decreased 20% (p:0.002) and when it occurred at 6h, the AUC_{0-6} decreased 50% (p<0.001).

Table 9 Multilevel linear model of the independent association of variables with rifampicin AUC_{0-6}

Variable	Proportional difference	CI	p-value
Fasting	1.14*	1.02-1.28	**0.021**
Tmax 4h	0.8	0.7-0.92	0.002
Tmax 6h	0.5	0.38-0.64	<0.001
Sex	1.26†	1.08- 1.48	**0.004**

Note: The model was considered based on the natural logarithm of the Cmax values. The proportional difference was calculated as the exponential of the coefficient obtained for each variable in the multilevel linear model. Interpretation of the proportional difference: * Rifampicin AUC_{0-6} in the fasting day was 14% higher than rifampicin AUC_{0-6} during the non-fasting day. † rifampicin AUC_{0-6} in female were 26% higher than rifampicin AUC_{0-6} in males.

5.2.2.2 Pharmacokinetics of isoniazid

Did isoniazid exposure at each time-point differ between fasted and non-fasted treatment days?

Overall, median serum isoniazid levels at two, four and six hours were 3.27, 1.96 and 0.92 mg/L respectively during the non-fasting day and 4.54, 1.19 and 0.75 mg/L during the fasting day.

Did isoniazid Cmax or AUC_{0-6} differ between fasted or non-fasted treatment days?

There were significantly difference in the Cmax at the non-fasting blood-sampling day (3.51 mg/L) compared with the Cmax at the fasting blood-sampling day (4.54 mg/L) (Wilcoxon Signed rank test p< 0.001). The AUC_{0-6} was 12.11 mg·h/L in the non-fasting day vs. 13.31 mg·h/L during the fasting blood-sampling day (p: 0.001).

Did effect of fasting on isoniazid Cmax or AUC_{0-6} differ between males and females?

The isoniazid *C*max in men (3.29 mg/L) was not different to isoniazid Cmax in women (4.41) (p: 0.08) in the non-fasting day and also in the fasting day (4.21 *vs*. 4.88 mg/L; p: 0.21). Similarly, there were not any difference in isoniazid AUC_{0-6} in males and females in both non-fasting and fasting day (Non fasting day: 12.08 *vs*. 1.65 mg·h/L, p:0.36; fasting day: 13.21 vs. 15.13 mg·h/L, p: 0.28). Isoniazid Cmax was not associated with intestinal parasites, the age group, or the body mass index (data not shown).

16A

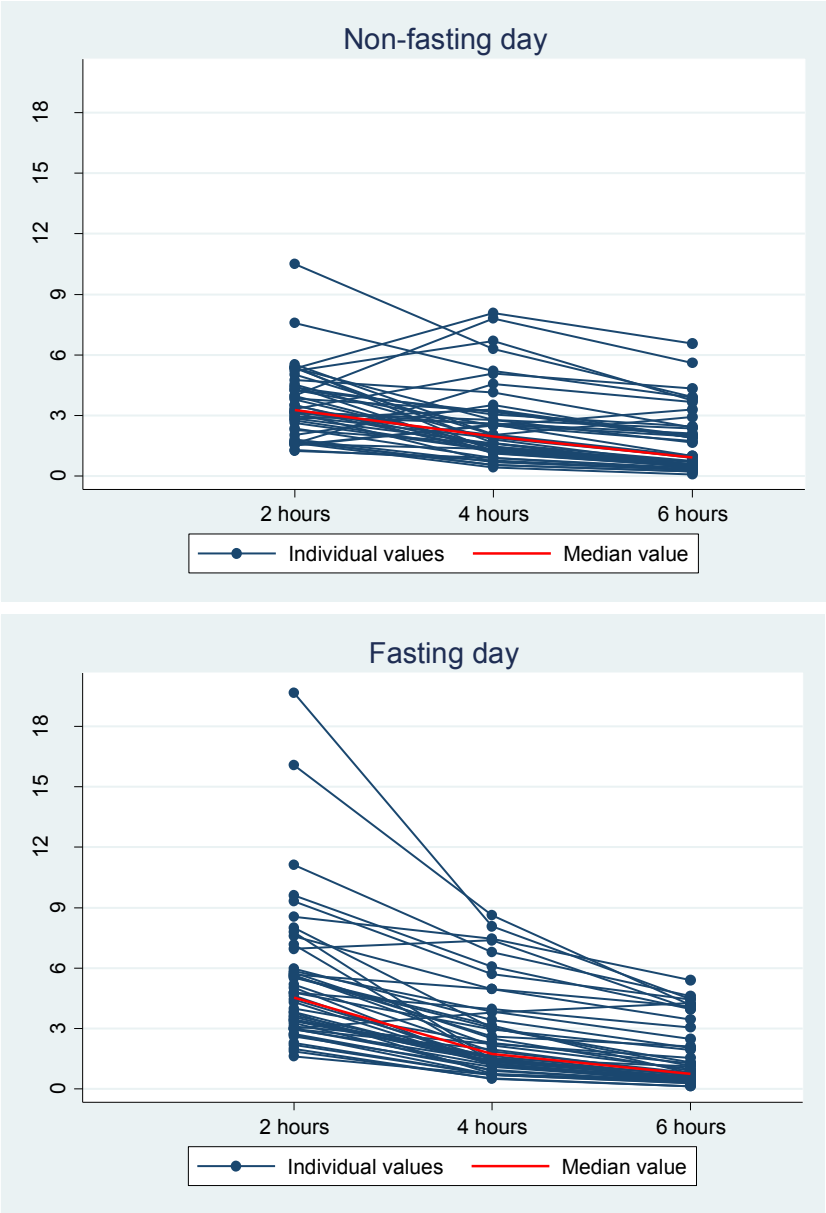


Figure 16 Pharmacokinetic levels of isoniazid in the non-fasting (15A) and fasting (1B) day

Effect of fasting on isoniazid Tmax

Tmax occurred at two hours in 80.43% and 95.8% of patients in the non-fasting and fasting day respectively; at 4 hours in 17.4% (non-fasting) and 2.08% (fasting) and at 6 hours in 2.17 (non-fasting day) and 2.08% (fasting day) of patients. Moreover, there were significant differences in the isoniazid Tmax in the non-fasting day compared with the fasting day. (p: 0.023, Wilcoxon Signed rank test).

Tmax was not associated with gender, age group, intestinal parasitic infection, or dose received (data not shown) in both non-fasting and fasting day.

Categorization of isoniazid Cmax: therapeutic *vs*. non therapeutic levels

In the non-fasting day, seven patients (15.22%) had *C*max values of <2 mg/L, and 39 (84.78%) had values that are regarded as acceptable normal levels. When the blood sample was taken during the fasting day, 3 (6.25%) had *C*max values of <2 mg/L, 45 (93.75%) had normal levels (>2 mg/L) (Fig. 17).

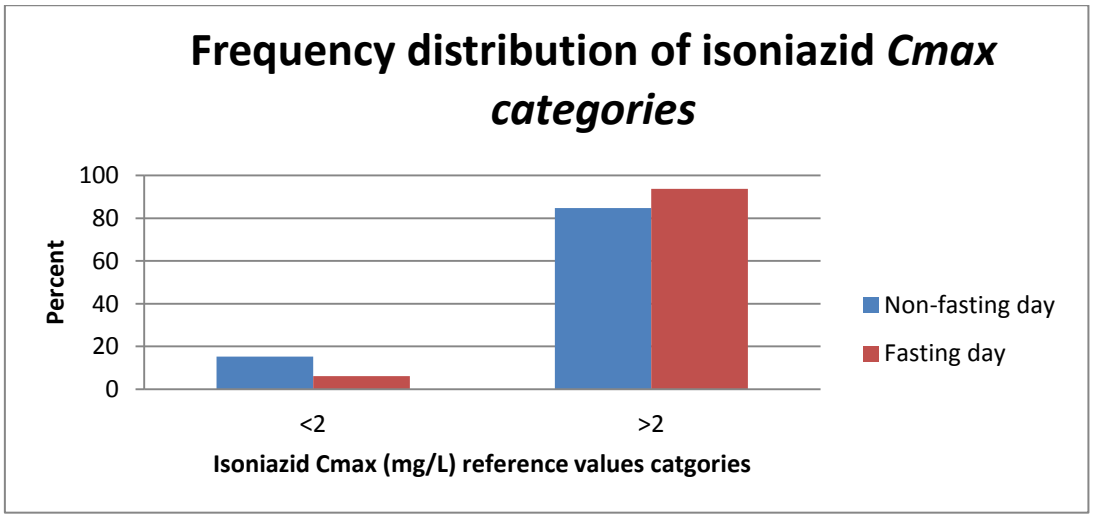


Figure 17 Frequency distribution of isoniazid Cmax categories during the non-fasting and the fasting day

Multivariate analysis

1. A multivariate analysis was performed through multilevel linear model, where the dependent variable was the logarithm of the isoniazid Cmax. We built a model to assess the independent effect of the gender, the isoniazid dose received and the effect of fasting during the drug intake on the isoniazid Cmax.

In this model, the isoniazid dose received had an effect upon the isoniazid levels (1.26, p:0.038). Isoniazid exposure in the fasting day was found to be 14% higher than those in the non-fasting day of females (Confident Interval: 1.02 – 1.28, p<0.001). The rest of the variables did not influence significantly the model.

Table 10 Multivariate regression model of the independent association of variables with isoniazid exposure (Cmax)

Variable	Proportional difference	CI	p-value
Fasting	1.14*	1.02-1.28	**<0.001**
Sex	1.26	1.08 – 1.48	0.176
Isoniazid dose	1.25	1.01-1.53	**0.038**
Note: The model was considered based on the natural logarithm of the Cmax values. The proportional difference was calculated as the exponential of the coefficient obtained for each variable in the multivariate model. Interpretation of the proportional difference: * Isoniazid Cmax in the fasting day was 14% higher than isoniazid Cmax during the non-fasting day. †			

2. A multivariate analysis was performed through multilevel linear model, where the dependent variable was the logarithm of the isoniazid AUC_{0-6}. We built a model to assess the independent effect of the gender, the isoniazid Tmax and the effect of fasting during the drug intake on the isonizid-AUC_{0-6}.

Isoniazid AUC_{0-6} in the fasting day was found to be 22% higher than those in the non-fasting day (Confident Interval: 1.09 – 1.38, p<0.001. When isoniazid Tmax occurred at 6h, AUC_{0-6} decreased 47% (p:0.013). The rest of the variables did not influence significantly the model.

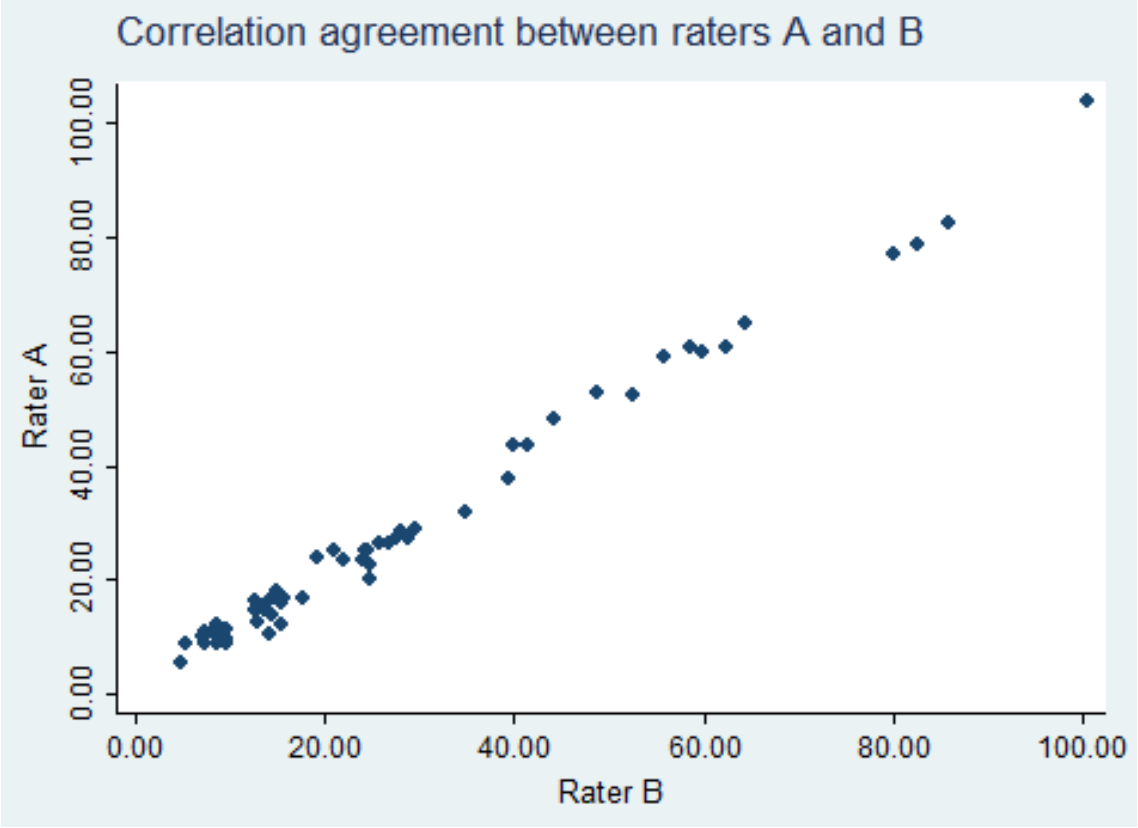
Table 11 Multivariate regression model of the independent association of variables with isoniazid exposure

Variable	Proportional difference	CI	p-value
Fasting	1.22*	1.09-1.38	**0.001**
Sex	1.25	0.91 – 1.71	0.171
Isoniazid Tmax 4h	0.96	0.73-1.27	0.786
Isoniazid Tmax 6 h	0.53	0.32-0.87	**0.013**
Note: The model was considered based on the natural logarithm of the Cmax values. The proportional difference was calculated as the exponential of the coefficient obtained for each variable in the multivariate model. Interpretation of the proportional difference: * Isoniazid AUC_{0-6} in the fasting day was 14% higher than isoniazid AUC_{0-6} during the non-fasting day. † When isoniazid Tmax occurred at 6h, Cmax decreased 47%.			

5.3 RESULTS OF STUDY No. THREE

5.3.1 CHEST RADIOGRAPHY (CXR) EVALUATION

CXR was only performed in 56 patients. ICC between the 2 observers was 0.923 (0.872-0.954, p<0.001) for the determination of the total area of the lungs (pixels) of each CXR and 0.977 (0.961-0.986, p<0.001) for the area of the lung affected. ICC was 1 when the presence of cavitation was evaluated. When the final composite score was determined, the ICC between the 2 raters was 0.995 (0.991-0.997, p<0.001) (figure 13). Kappa coefficient for IOA of the score was 0.86 (p<0.001) and when the proportion of lung affected was evaluated, kappa coefficient was 0.9 (p<0.001).



Ralph score: proportion of total lung affected (%) + 40 if cavitation

Figure 13 Intraclass correlation agreement between rater A and B.

6. DISCUSSION

The fact that pharmacokinetics of TB drugs can be a factor in the outcome of TB disease has been widely discussed. A pharmacokinetic interaction during the absorption, transport distribution or elimination may modify the drug's effect. This can lead to treatment failure or relapse and death from TB in specific cases[101]. Other possible sequelae are prolonged infectiousness and development of acquired drug resistance[102]. Therefore, in selected groups of patients, impaired pharmacokinetics must be suspected when patients early fail to respond to directly-observed therapy.

We will discuss different aspects of PKs' results of the studies as well as the treatment outcome.

6.1 RIFAMPICIN AND ISONIAZID DOSAGE

In the first study, a major finding was that most patients during the continuation phase received a weight adjusted biweekly dose of isoniazid below the PNTP-recommended 15-mg/kg/day dose[103]. This was a particular problem in TB patients with DM who had a higher BMI and also in men compared with women, but we were unable to determine whether the lack of dose adjustments to account for weight gain during treatment may also have contributed. These observations have since led the PNTP to change the maximum biweekly isoniazid dose in the continuation phase from 800 mg to 900 mg and to reemphasize the importance of weight-based dosing (See section 4.1).

In the second study, weight adjusted rifampicin and isoniazid dose was accorded to the updated PNTP recommended dosages (See section 4.2).

6.2 FACTORS ASSOCIATED WITH RIFAMPICIN PK

6.2.1 STUDY No. ONE

The key finding of this research study, carried out under real-life field conditions and not in the controlled environment of a dedicated PK unit, is that measured serum rifampicin levels in patients receiving directly observed TB treatment in the Peruvian NTP were highly variable and very frequently well below what is conventionally regarded as a therapeutic range.

Cmax – Tmax – AUC_{0-6}

We observed very low levels of rifampicin at 2 h in 68.6% of patients, which is in accordance with data from other studies[98,104]. Moreover, 36 patients (34.3%) had undetectable levels at 2 h, when the peak concentration after oral administration theoretically occurs[77,105]. Wilkins *et al*. previously reported considerable inter-individual variability in rifampicin pharmacokinetics in South Africa and suggested that highly variable rates of absorption could significantly impact the Cmax, since, as further confirmed by our data here, slower absorption leads to lower peak plasma concentrations[106].

In this study, 61 patients had higher levels of rifampicin at 6 h than at 2 h, which we attributed to a delay in absorption; thus, the Tmax varied significantly. Aside from inter-individual variability, we have explored other potential explanations outlined below. A strength of this study was its real-life execution in health centres; thus, samples were taken from patients doing what they do every day and not under the tightly controlled conditions of a dedicated PK ward. As such, despite the known effect of food upon the pharmacokinetics of rifampicin (and other TB drugs)[88,101,107], many patients did not fast before taking treatment; indeed, fasting is not a feature of the Peruvian NTP guidelines. In the small subgroup of patients for whom oral intake data were available (48 of 105), we could not demonstrate any effect of food on the Cmax or Tmax.

The main limitation of this work was the inability to calculate the 0- to 6-h area under the PK curve (AUC_{0-6}). We sampled only two time points, 2 and 6 h, for pragmatic and logistic reasons and because this approach was previously successful. However, the real Cmax might have occurred between the 2- and 6-h samplings, and furthermore, the unexpected finding of so many measurements below the limit of detection and the fact that a considerable proportion of patients had their Tmax at 6 h rendered AUC_{0-6} calculations futile.

Co-morbidities

The first study was designed to determine to what extent a DM or HIV co-morbidity might impair rifampicin pharmacokinetics, but neither of these conditions had any demonstrable effect on either the rate of rifampicin absorption (Tmax) or the magnitude of absorption (Cmax).

Regarding DM, other studies have shown conflicting results. One study found that rifampicin exposure was 2-fold lower in diabetic than in non-diabetic TB patients during the continuation phase of treatment[77]. Nevertheless, a second study conducted on the same setting to patients during the intensive phase of the treatment, concluded that DM does not alter the pharmacokinetics of rifampicin and isoniazid[108].

Another study showed that rifampicin and isoniazid serum concentrations decreased by 50% ($p<0,05$) in diabetic pulmonary TB patients compared with TB patients without co-morbidity[109]. However, this study collected only one blood sample two hours after the drug intake and therefore a possible delayed absorption has not been considered. Finally, a more recent study developed a nonlinear mixed-effect model (NONMEM) to determine the population PK parameters of rifampicin and the effect of DM status in patients with TB and the rifampicin concentrations were predicted to be lower in patients with DM [110].

In addition, if DM is confirmed to be an independent risk factor for treatment failure [111], our data suggest that this is not related to deranged pharmacokinetics of rifampicin, nor is it associated with hyperglycaemia, although the limited pharmacokinetic analysis might hamper the assessment of the effect of DM on the pharmacokinetics of rifampicin.

Regarding HIV, it remains controversial whether a significant malabsorption of antimycobacterial drugs occurs in HIV patients[75,79,81,82,112] and, if so, whether this is associated with treatment failure or the emergence of drug resistance. Although a crude analysis revealed marginally reduced Cmax values in HIV patients (data not shown), after stratifying data by the Tmax, no associations remained between the rifampicin level and HIV status in either fast or slow absorbers. HIV-related achlorhydria, HIV enteropathy[113], and opportunistic infections of the intestinal tract were reported previously[101,114] to be risk factors for a poor treatment outcome; we did not explore these risk factors other than in the search for intestinal parasites. Moreover, according to McIlleron *et al*, level of immunosuppression and concomitant antiretroviral therapy has little effect on the concentrations of the antituberculosis agents[115].

Finally, some studies suggest that in HIV-positive patients, lower plasma concentration might be associated with treatment failure (despite a proper adherence to DOT) with the subsequent development acquired resistance [102] and with a greater risk of tuberculosis recurrence among HIV infected patients with more advanced immune suppression [116–118].

Gender

Men received a lower mg/kg dosage and had lower maximum rifampicin levels than women. However, even after adjusting for the rifampicin dosage received in the multivariate analysis, men had significantly lower plasma levels than women, a finding reported previously[98,119] and likely due to an increased volume of distribution [120].

Intestinal parasitosis

No association was found between rifampicin PK and intestinal parasitosis, which affected almost 10% of participants, which does not support the hypothesis that intestinal parasitosis might affect TB treatment outcomes through a PK-related mechanism [94].

Drug formulations

The absorption of orally administered drugs can be modified by the nonspecific adsorption of drugs to excipients in formulations [101]; a study in South Africa documented a poor relative bioavailability of rifampicin from some fixed-dose combinations[121]. It was not clear whether bioequivalence studies had been performed for the two generic preparations of rifampicin in use[122]; although data were incomplete, we were unable to demonstrate a difference in the PK results for each preparation.

6.2.2 STUDY No. TWO

Cmax – Tmax – AUC

In the second study, and as predicted in the first study, amongst those who took drug with food, we observed a delay in the rifampicin absorption and the median Tmax occurred at 4 hours instead of 2 hours. However, when the blood sampling was done on an empty stomach, an absorption delay was not observed and the Tmax occurred at two hours in most patients.

A limitation of the first study was that the effect of the food on the Cmax or Tmax could not be demonstrated since most patients did not fast before drug intake. For this reason, the design of the second study included two blood-sampling days and in one of these days, patients were asked to fast before the drug intake. Thus, this intra-individual PK analysis that excluded the inter-individual variability, demonstrated the interaction of the food during the drug intake in the absorption delay (Tmax) and also in the AUC_{0-6}, which has been corroborated in the multivariate analysis. As it has been suggested before and further confirmed by our data here and in the first study, slower absorption leads to lower plasma concentrations [106].

Overall, the Cmax was marginally higher when compared with the Cmax of the first study irrespective of the rifampicin had been ingested with food or not. In this study, when people ingested rifampicin with food, we observed subtherapeutic or low levels in more than half of patients although very low levels (<4mg/L) were only observed in less

than 10% of patients, which is a substantially lower percentage compared with data observed in the first study. One possible reason to explain this difference is the fact of using only two time-points in the first study, which may have not captured peak concentrations occurring between the two blood draws. Thus, the real value of Cmax may have been underestimated.

Gender (see section 4.2)

In this study, men had lower levels of rifampicin Cmax and also a lower rifampicin AUC_{0-6} compared with women when rifampicin was taken with food. Although this effect was not consistent when the blood sample was taken on an empty stomach, in the multivariate analysis the gender influenced the pharmacokinetics of rifampicin regardless the fact of fasting during the blood sampling. As suggested in other studies, these differences in the PK parameters should be considered for further dosing recommendations[123].

6.3 FACTORS ASSOCIATED WITH ISONIAZID PK

Isoniazid usually shows very high inter-individual pharmacokinetic variability, with a multi-modal distribution of exposure [124–126].

6.3.1 STUDY No. ONE

In this study, we aimed to study the isoniazid PK under real-life conditions in the PNTP using sparse PK sampling during daily and twice-weekly dosing. The range of isoniazid doses included in the study was wider than in most published PK studies and clearly demonstrates the linearity of isoniazid PK with weight-adjusted doses. With the two dose regimens, the isoniazid PK was highly variable, with >30% of patients failing to exceed a Cmax deemed very low compared with those in other PK studies.

Cmax - Tmax – AUC_{0-6}

The sparse sampling scheme limited our ability to detect changes in the rate of absorption reflected in the Tmax or Cmax. If such altered absorption exists, it did not seem to influence the overall AUC_{0-6}, notwithstanding the reduced precision of calculating an AUC using two time points versus more enriched time point sampling.

Plasma exposure as measured by the median $AUC_{0–6}$ was comparable with the median AUC_{0-12} described for patients with favorable treatment outcomes in two Tuberculosis

Trials Consortium (TBTC) studies (28 mg·h/liter in “study 22” [61] and 48.8 mg·h/liter in “study 23”[102], which used the same biweekly dosing regimen.

Co-morbidities

Neither DM nor HIV independently influenced the isoniazid PK. The DM population in the twice-weekly dosing group showed lower isoniazid exposure, but this was explained by the higher BMIs.

NAT2 (N-arylamine acetyltransferase)

The pharmacokinetic phenotype of isoniazid is determined by polymorphisms at the N-arylamine acetyltransferase (NAT2) locus[127], with alleles conferring a “slow” acetylator phenotype associated with 4-fold greater exposure compared with “rapid” acetylators [95,128].

We did not obtain NAT2 genotype or metabolite data to accurately classify individuals by acetylator status. However, the population distribution of isoniazid half-lives was clearly bimodal. Even though the underlying distribution of acetylator phenotype is, in fact, trimodal [95], our data did not support a clear differentiation between intermediate and slow acetylators.

More than half of the patients were predicted to be fast acetylators, which may have also contributed to low and variable isoniazid exposure.

Little information is currently available on acetylator status in Latin America, especially in Peru, where the *4 allele associated with the fast acetylator phenotype, and which is overrepresented in Asia, may be common[129]. Future studies should address this issue with genotyping or metabolite data.

6.3.2 STUDY No. TWO

Tmax occurred at two hours even the blood-sampling was not taken on an empty stomach although the PK parameters (Cmax) were higher in the fasting blood sampling, which is in accordance with other studies that suggest that food causes an absorption delay and also reduces the Cmax[105].

It has been suggested that Tmax for isoniazid may sometimes occur earlier than 2 hours and we would not have captured this as our earliest sampling time point was 2 hours.

The multivariate analysis also demonstrated the effect of fasting on increasing the exposure to isoniazid.

Gender

In both univariate and multivariate analysis AUC_{0-6} and Cmax for isoniazid did not differ by gender in either fasted or non-fasted state.

As with rifampicin, men had also significantly lower levels than women.

6.4 THERAPEUTIC PK PARAMETERS AND RESPONSE TO TREATMENT

Efficacy targets for PK parameters for anti-TB drugs have historically been based on reference therapeutic ranges derived from achieved PK alone [73]. The relevance of these to short- or long-term outcomes remains unclear.

The normal pattern for TB drug serum concentrations shows the 2-h values as substantially higher than the 6-h values. As occurred in the first study, should the 2- and 6-h values be similar, often somewhat below the expected 2-h ranges, or should the 6-h values be higher than the 2-h values, delayed absorption is likely occurring [105]. Then, it is also possible that the peak concentrations occurred between the two blood draws. This is what was demonstrated later in the second study when more than half of patients had the rifampicin Tmax at 4 hours although it did not occur in the case of isoniazid.

Rifampicin absorption may be the most variable among the TB drugs[130] , being reduced or delayed by high-fat meals or even when elaborated with isoniazid and pyrazinamide as fixed-drug combination. Rifampicin clearly exhibits concentration-dependent killing, and higher doses might be tolerable and more efficacious[105].

In the first study, there was a widespread perturbation of what is considered to be "normal" rifampicin pharmacokinetics across all patient groups[73]. Specifically, two-thirds of patients had delayed rifampicin absorption, with higher levels at 6 h than at 2 h post-dosing, and for only one-quarter of patients was a measured rifampicin serum level (whether at 2 or 6 h) above the 8-mg/L threshold regarded as necessary for therapeutic efficacy. Moreover, most cured patients had also Cmax levels below 8 µg/mL.

In the second study, we have also observed "subtherapeutic" levels of rifampicin in less than 33% of patients although only one patient was finally reported to have a bad treatment outcome.

Therefore, further investigation is needed to ascertain whether a review of the purported normal rifampicin Cmax of >8 µg/mL is warranted. However, some studies have demonstrated that an increase in the dose of rifampicin led to a better treatment outcome [131]. Therefore, if we assume a higher median concentration in those patients who received a higher dose, we could consider that there is a correlation between the concentration and the treatment outcome.

Isoniazid is generally quickly absorbed with a Cmax occurring 1-2 h post-dose when given on am empty stomach. If only isoniazid is measured, 1- and 4-h concentrations may effectively capture Cmax and most cases of delayed absorption. However, since isoniazid is routinely given with other agents that are somewhat more slowly absorbed, 2- and 6-h post-dose samples are preferred[105].

In the first study, although the Cmax was low in up to 30% of the participants, the AUC_{0-6} values were comparable with those of other studies and were not associated with poor outcomes. In particular, despite high inter-individual variability in exposure, twice-weekly weight-adjusted dosing of isoniazid appears quite robust with respect to important patient factors under program conditions.

However, in the second study most patients had normal Cmax values and in the subanalysis performed with the 37 patients followed-up, there was only one case of poor treatment outcome. However, the study was not sufficiently powered to definitively prove whether poorer outcome was associated with rifampicin or isoniazid exposure.

There were several studies in the 1960s that related the treatment outcome to PK and particularly to isoniazid concentrations [132,133]. Gangadharam *et al*. showed that the therapeutic efficacy increased above a critical peak concentration of "about 3 µg/ml" of isoniazid [133]. Another study supporting these data showed that early bactericidal activity (EBA) levelled off at a 2-h isoniazid concentration between 2 and 3 g/ml[74].

Weiner *et al*. also reported low isoniazid concentrations associated with poorer treatment outcome in patients who received once-weekly isoniazid with rifapentine but not in those who received twice-weekly isoniazid with rifapentine[61], which might serve as a surrogate for what should be expected if there was any noncompliance during the daily therapy. On the other hand, most studies of longer-term outcomes have not reported associations with isoniazid PK parameters; instead, pyrazinamide or rifampicin is usually implicated[56,66,100,119,134,135]. In this study, cut-offs suggested in the literature were not associated with poor outcomes. However, the relationship of the isoniazid AUC with drug effect (at least as measured by early bactericidal activity) is well defined[129], which suggests that the variability in PK that we observed might be of clinical significance, especially during intermittent dosing.

In our study, it seems that lower serum concentrations, particularly with rifampicin can be effective therapy in most patients. However, we could not accurately measure the time to response or in other words, if there were patients that required more than the standard 6-months schedule therapy. In this regard, there is no clear guidance on “how low can you go” since it is likely to vary from patient to patient and cannot be defined at the outset of therapy [105].

In addition, we could not accurately measure the effect of how the combination therapy might have positively influenced the treatment outcome. Although this synergistic effect can potentially be measured through a microdilution checkerboard assay [136], drug susceptibility testing is done individually for each drug [137]. Thus, any effect of a low rifampicin or isoniazid concentration might be overcome by the effect of the other agents and thus not directly influence treatment outcome.

Finally, a perennial and inherent problem with TB drug PK analyses is unpicking whether plasma rifampicin levels, defined as Cmax or (for preference) total exposure measured as AUC, are the crucial determinant of drug efficacy at a cellular level, and if so, what the minimum drug exposure (measured in plasma) needs to be in order to have a high probability of efficacy. Evidence for poorer treatment outcomes associated with impaired pharmacokinetics is, perhaps surprisingly, somewhat thin and less persuasively demonstrated for rifampicin[98] than for pyrazinamide[56].

6.5 TREATMENT OUTCOME

Regarding treatment outcome, in the first study, a subanalysis was undertaken with a limited number of patients that were further followed-up. In this subanalysis, we only included 41 drug susceptible patients, excluding patients diagnosed with multidrug-

resistant TB, patients who either abandoned treatment or did not properly complete treatment, and others who lacked a microbiologically confirmed diagnosis. However, we agree that our study was not designed to relate the treatment schedule strategy to treatment outcome and that larger studies with long-term follow-up are required to provide relevant data in this respect.

In the second study, a limited number of patients (37 out of 60 recruited) were followed-up for six months. Patients with mono-drug resistance or MDR were excluded from this analysis as well as those who were diagnosed with HIV, abandoned the treatment or had treatment modified due to adverse events. Only one of these 37 patients suffered early relapse.

In this under-powered subanalysis, the treatment outcome was neither influenced by impaired pharmacokinetics (rifampicin or isoniazid) or by difference in the intake of TB drugs with food. However, the small number of poor treatment outcomes means that this could represent a type II error.

6.6 THERAPEUTIC DRUG MONITORING

Although therapeutic drug monitoring (TDM) is neither widely used nor recommended during TB treatment, it is widely held that abnormal TB drug pharmacokinetics may adversely influence outcomes, with potential consequences including treatment failure, relapse or death, prolonged infectiousness, and the development of acquired drug resistance[101,102]. In addition, TDM might contribute not only to the identification patients with low levels of rifampicin or isoniazid but also to a shrinking the time to response and also the duration of treatment[138]. TDM provides objective information for the clinician to make informed dosing decisions.

The first study with a pragmatic study design based on two sampling points, two and six hours after drug intake, reflected the real world approach taken in this study, sampling patients attending a government clinic rather than in the controlled setting of a pharmacokinetic study suite. This sampling strategy has been frequently used in the past[105] and there is data to suggest that the peak serum concentration (Cmax) occurs between one to two hours post-dose when isoniazid is given on an empty stomach[73]. If only isoniazid is being measured, a two-sample strategy with estimation of one and four hour concentrations may effectively capture both the Cmax and the majority of delayed absorption as we observed in the second study. However, isoniazid is usually administered with other drugs which are somewhat more slowly absorbed (e.g. rifampicin). In such cases, a two and six hour post-dose sampling

strategy chosen in the first study facilitates the analysis of both agents and it seems reasonable and practical. A more intensive pharmacokinetic sampling will better capture the true Cmax particularly for rifampicin parameters values as demonstrated in the second study. However, the logistical demands of this approach compromised study recruitment in our community clinic field sites, limiting the power of the analysis.

Limitations of TDM

Regarding the interpretation of the TDM results, it is very complex to define the endpoints of thresholds of “therapeutic” since the disease in humans is much more complex than in vitro models and more variable than the situation produced in animal models[105]. In humans, there are different strains of TB causing infection and there is also re-infection, latency, relapse, varying immune-response and co-morbidities. All these variables that can be controlled in vitro models cannot be quantified in humans. Thus, it is acceptable that the responses to treatment in humans also cover a wide spectrum.

Another drawback is that sometimes conventional blood sampling cannot be performed, especially in areas with limited resources. A possible alternative is to use dried blood spots, where blood samples are collected and dried before storage and transportation. Dried blood spots are easier to transport and offer cost savings[139]. However, this area still is evolving and requires further research and validation. Also, when using dried blood spots, drug concentrations are being measured in whole blood, and not in plasma[71]. This technique has been applied successfully for some of the TB drugs, like rifampicin and linezolid [140–142].

6.7 EFFECT OF FOOD ON PK OF RIFAMPICIN AND ISONIAZID AND TREATMENT OUTCOME

It is likely our data may underestimate the true Cmax and AUC though there is no reason to believe this would differ between the fasting and non fasting groups. However, one factor that may delay the time at which Cmax occurs (Tmax) is to take the drug with food, in particular high-fat meals[143]. Thus, it is recommended that isoniazid should be given on an empty stomach. In the first study, we were unable to adequately control or reliably measure the concomitant food intake of all the patients and this fact may have influenced the final results. While we regarded the conduct of the study under real-life circumstances as being important and a strength of the data, we were unable to adequately control and reliably measure concomitant oral intake for

all participants. Gastrointestinal upset is common in TB patients, and many gain relief by taking medicines with food or juice; a future study might usefully examine detailed PK curves for the same patient on consecutive days under different conditions of oral intake.

The second study was designed with three time-points: two, four and six hours after the drug intake and in two different days (one of them being fasted before the drug intake) in order to provide more accurate PK data. The results of this study demonstrate that rifampicin and isoniazid Cmax are reduced when the drugs are given with meals as has been shown in other studies[144]. Moreover, the time of isoniazid Cmax (Tmax) is delayed. For this reason rifampicin and isoniazid are recommended to be given on an empty stomach whenever possible.

However, few studies have evaluated if patients dosing the TB drugs with meals are associated with treatment failure or early relapse. The US Public Health Service TB Trial 22 reported that patients receiving medication under fed conditions were significantly associated with treatment failure or relapse although patients were receiving isoniazid and rifapentine instead of RIF[61]. It is well known that rifapentine pharmacokinetics are highly food-dependent. In our study, we could not demonstrate if taking the drugs with meals is associated with a poor response to treatment. Indeed, most patients did not fast most days during the drug intake in both intensive and maintenance phase and we accounted only one case of poor treatment outcome. This suggests that it may be other factors influencing the treatment response. As a way of example and as mentioned before, the effect of the combination therapy or the unknown determinants of drug efficacy at a cellular level could influence positively the treatment outcome and despite low serum rifampicin or isoniazid concentrations, these facts may contribute to a good treatment outcome.

7. CONCLUSIONS

1. Rifampicin and isoniazid dosage

   Since rifampicin and isoniazid exposure may be affected by weight-adjusted dose, rifampicin and isoniazid dose should be adjusted by weight and the Peruvian National TB program should reemphasize the importance of weight-based dosing throughout the duration of the treatment.

2. Rifampicin pharmacokinetics and TB co-morbidity

   In the first study, most of this Peruvian study population exhibited rifampicin pharmacokinetics different from those conventionally reported, with delayed absorption and low plasma concentrations, independent of the presence of HIV or DM co-morbidity.

3. Isoniazid pharmacokinetics

   The variability in isoniazid exposure was driven largely by weight-adjusted dose, whereas important co-morbidities, such as DM and HIV, had no demonstrable additional impact on isoniazid PK. Although the Cmax was low in up to 30% of the participants, the AUC_{0-6} values were comparable with those of other studies and were not associated with poor outcomes.

4. Food and pharmacokinetics of rifampicin and isoniazid

   Rifampicin in particular and also isoniazid pharmacokinetics (both Cmax and AUC_{0-6}) were significantly affected by the intake of the drug with food.

5. Rifampicin and isoniazid pharmacokinetics and treatment outcome

   A clear relationship between the pharmacokinetics parameters and treatment outcome was not demonstrated.

   Weight-based twice-weekly dosing remains a pharmacologically appropriate strategy in this context, and this dosing strategy appears to be quite robust with respect to the important potentially influential patient factors studied here, at least in the Peruvian National TB program.

6. Therapeutic drug monitoring

   Therapeutic drug monitoring (TDM) based on targets for pharmacokinetics parameters for TB drugs is currently neither widely used nor recommended during TB treatment. Further investigation is needed to ascertain whether a review of the purported normal or therapeutic rifampicin and isoniazid pharmacokinetics is warranted.

7. Other factors associated with pharmacokinetics of rifampicin and isoniazid

   Intestinal parasitosis did not affect both rifampicin and isoniazid pharmacokinetics.

   Rifampicin exposure was significantly lower in men compared to women.

   The acetylators status, may have contributed to low and variable isoniazid exposure

SUMMARY (SPANISH VERSION)

INTRODUCCION

La mayoría de pacientes con tuberculosis tienen una respuesta terapéutica adecuada y de hecho la mayor causa de fracaso terapéutico y recaída se deben a una mala adherencia al tratamiento. Sin embargo, un cierto porcentaje con mala respuesta al tratamiento se debe a una farmacocinética alterada de los fármacos antituberculosos. De hecho hay estudios que asocian la alteración farmacocinética con el desarrollo de resistencias a los fármacos antituberculosos.

Hay varios factores que se han asociado con una alteración farmacocinética de los fármacos antituberculosos, incluyendo el género, la edad, la ingesta de fármacos con la comida, la interacción con otros fármacos, y ciertas co-morbilidades como la Diabetes Mellitus o la enfermedad por VIH.

Aunque los datos sobre la farmacocinética de los fármacos antituberculosos en relación al efecto de la comida y de los antiácidos sobre la absorción gastrointestinal de los fármacos son limitados, la biodisponibilidad de los fármacos antituberculosos, en especial de rifampicina e isoniacida puede verse disminuida con la ingesta concomitante de comida, lo que podría llevar a una disminución del efecto del fármaco y por tanto a una peor evolución clínica de la enfermedad. Por este motivo, se recomienda la administración de rifampicina e isoniacida en ayunas. Existen muchos estudios que han evaluado la ingesta de rifampicina y de isoniacida en ayunas desde un punto de vista farmacocinético pero no se ha evaluado de forma tan exhaustiva el efecto de la ingesta de la medicación antituberculosa en ayunas en la respuesta al tratamiento.

OBJETIVOS

El objetivo del primer estudio es evaluar los niveles farmacocinéticos de rifampicina e isoniacida en una población peruana en tratamiento para tuberculosis así como su respuesta al tratamiento y compararla con un grupo de pacientes con co-morbilidades (DM y VIH).

El objetivo del segundo estudio es en un grupo de pacientes peruanos en tratamiento con antituberculosos de primera línea, evaluar el efecto de la comida durante la ingesta de los fármacos antituberculosos en la farmacocinética de los mismos y en la respuesta terapéutica al final del tratamiento y al cabo de 6 meses.

MÉTODOS

Se han desarrollado dos proyectos de investigación y un sub-estudio en el marco del segundo proyecto de investigación. Los estudios se desarrollaron en Perú, en los "Laboratorios de Investigación y Desarrollo" de la Universidad Peruana Cayetano Heredia. El reclutamiento de los pacientes se realizó en las Unidades de "Sintomático respiratorio" de varios centros de salud pertenecientes a la Dirección de Salud de Lima II (DISA II) y en la Unidad de tuberculosis del Hospital Dos de Mayo de Lima (DISA V).

En ambos estudios se requirió la aprobación de los comités de ética de la London School of Hygiene and Tropical Medicine, Hospital Clínic (segundo estudio), Universidad Peruana Cayetano Heredia, Hospital Dos de Mayo y el comité de ética de la DISA II.

En el primer estudio desarrollado de Junio a Diciembre del 2009, fueron invitados a participar (i) pacientes diagnosticados de tuberculosis, (ii) en tratamiento de primera línea (por tanto incluyendo isoniacida y rifampicina) desde hace más 15 días y (iii) en el marco del programa de "tratamiento directamente observado" que se ejecuta en el programa nacional de Tuberculosis de Perú (PNTP). Además de un cuestionario epidemiológico se recogieron muestras de sangre (a las 2 y a las 6 horas tras la ingesta de la medicación) para el análisis farmacocinético así como una muestra de heces para el examen de parásitos en heces.

En el segundo estudio desarrollado en Lima de Enero a Diciembre de 2012, fueron invitados a participar pacientes con tuberculosis pulmonar bacilíferos que entraron en el programa nacional de tratamiento del PNTP. Además del cuestionario epidemiológico, se les entregó a todos los participantes del estudio, un dietario donde anotaban diariamente si ayunaban o no con la ingesta de la medicación, se les realizó una Rx de tórax, y en los días 30 y 60 tras el inicio de la medicación, se les recogió una muestra de esputo y se les recogió una muestra de sangre a las 2, 4, 6 horas tras la ingesta de la medicación. Uno de los 2 días en que se recogieron las muestras de sangre al paciente (el día 30 o el 60), seleccionado de forma aleatoria, se solicitó a los pacientes que ayunaran previa y posteriormente a la toma de la medicación; de este modo se garantizó un día de la ingesta de la medicación en ayunas.

Procesamiento de las muestras: Las muestras fueron centrifugadas y almacenadas a -70º hasta su envío a la "Liverpool School of Tropical Medicine donde se realizó el análisis farmacocinético a través de técnicas de cromatografía.

Análisis de datos: Para cada paciente se estimó la concentración máxima (Cmax) como la mayor de las medidas de cada paciente y la Tmax como el tiempo en el que la

Cmax ocurrió. Las características generales y demográficas se analizaron con el test Chi-cuadrado para la comparación de proporciones y con el test de T-Student para la comparación de variables cuantitativas. Variables no distribuidas de forma normal se analizaron con el test de Mann-Whitney. Se realizó en ambos casos análisis multivariado a través de regresión linear y modelos multinivel lineares. Los datos se introdujeron en una base de datos EpiData y fueron analizados con STATA, versión 10 y 13.

Para evaluar la radiografía realizada a todos los individuos incluidos en el estudio, se utilizó el score de Ralph en el que se utiliza la ecuación: porcentaje de pulmón afectado + 40 (si existe cavitación). El estudio 3, utiliza el software GIMP para diseñar y validar un método por el que poder medir el Ralph score de una forma más exacta y objetiva a través de la medición en pixeles del área seleccionada de una imagen.

RESULTADOS

Estudio 1

En el primer estudio se reclutaron 113 individuos de los que 26 tenían además DM y 29 tenían VIH. Los pacientes diabéticos eran mayores y tenían un índice de masa corporal mayor que los pacientes sin co-morbilidad ($p<0.001$), mientras que los pacientes con VIH eran mayoritariamente varones ($p<0.001$). La dosis de rifampicina fue significativamente inferior en varones (9.5 *vs*. 10.5mg/Kg/día, p:0.004). La dosis de isoniacida también fue inferior en pacientes diabéticos (11.6 *vs.* 13.3 mg/Kg/día, p=0.015) y en pacientes varones (12.22 *vs*. 13.48 mg/Kg/día, p= 0.008). En la fase de mantenimiento del tratamiento, la dosis de isoniacida media (12.8 mg/Kg/día), estaba por debajo de la dosis recomendad por el PNTP (15 mg/kg/día) ($p<0.001$).

Datos de rifampicina

El análisis farmacocinético mostró que la Tmax ocurrió a las 6h (grupo 1: retraso en la absorción) en vez de a las 2 horas (grupo 2: no retraso en la absorción) en el 62% de los pacientes. El hecho de tener un retraso en la absorción no se relacionó con tener co-morbilidad ni con el género, la edad, o la parasitosis intestinal. La media geométrica de Cmax del primer grupo (5.0 mg/L) fue significativamente menor que la del segundo (3.8 mg/L) ($p = 0.05$). Del mismo modo, la Cmax fue significativamente inferior en hombres que en mujeres (3.3 *vs.* 6.3 mg/L; $p < 0.001$). Tanto en el grupo con retraso en la absorción como en el que no la tenía, la Cmax no se asoció con co-morbilidad ni tampoco con la dosis de rifampicina recibida. El 34% de los pacientes tenían niveles indetectables de rifampicina a las dos horas. El análisis de regresión lineal mostró que

el sexo femenino (p<0.001) y la Tmax (p:0.12) fueron variables independientes que se correlacionaron con los niveles farmacocinéticos de rifampicina.

Datos de isoniacida

La Cmax y el área bajo la curva de las 0 a las 6 horas (AUC_{0-6}) de isoniacida fueron 2.77mg/L y 9.71 mg·h/L cuando la isoniacida se administró diariamente (5mg/Kg/día) y 8.74 mg/L and 37.8 mg·h/L respectivamente cuando se administró 2 veces por semana (15mg/Kg/día). No hubo diferencias en la Cmax con respecto al género o la co-morbilidad. La ingesta de la comida con la medicación se asoció débilmente con niveles disminuidos de la Cmax durante la fase de mantenimiento. El 34% de los pacientes durante la fase intensiva y el 33.3% de pacientes en la fase de mantenimiento no alcanzaron los niveles terapéuticos de Cmax. Sin embargo, niveles disminuidos de Cmax no se asociaron a una peor respuesta al tratamiento.

Estudio 2

Sesenta pacientes fueron incluidos en el estudio con una edad media de 32.7 años, siendo el 56% hombres. Las dosis medias de isoniacida y rifampicina fueron acordes a las recomendadas por el PNTP. Treinta y siete pacientes fueron evaluados al finalizar el tratamiento y 6 meses después y sólo uno de ellos tuvo una mala respuesta terapéutica. Los pacientes mayoritariamente tomaron la medicación con comida (una mediana del 96.5% de las veces) tanto en la fase intensiva del tratamiento como en la fase de mantenimiento (96% de las veces).

Datos de rifampicina

Se observó una diferencia en la Cmax (6.59 mg/L) en el día que no ayunaron comparada con el día de ayuno (7.02 mg/L) (p: 0.054); así como en la AUC_{0-6} 24.31 *vs.* 28.64 mg·h/L (p:0.002). La Cmax de rifampicina fue inferior en varones (6 *vs*. 8.3 mg/L; p: 0.035) en el día de ayuno pero no en el día de no-ayuno (6.73 *vs.* 7.55 mg/L; p: 0.09). Hubo diferencias significativas en la Tmax en el día de ayuno (mediana 2h) en comparación con el día de no ayuno (mediana 4h), (p:0.005). El Tmax no se asoció con el género, la edad, la parasitosis intestinal o la dosis recibida. En el análisis multivariado realizado a través de modelo de regresión multinivel, donde la variable dependiente era el logaritmo de la Cmax de rifampicina, el Tmax no tuvo un efecto en los niveles de rifampicina pero el ayuno incrementó los niveles de Cmax un 15% (p<0.036) y el género (mujeres) un 20% (p: 0.027). Cuando la variable dependiente fue la $AUC_{0-6,}$ el ayuno también incrementó los niveles de AUC_{0-6} un 14% y la AUC_{0-6} de las mujeres fue un 2% mayor en comparación a la de los hombres (p:0.002). Un retraso significativo en la absorción supuso una disminución de los niveles de AUC_{0-6}

de un 20% cuando el Tmax ocurrió a las 4 horas (p<0.001), y de un 50% cuando el Tmax ocurrió a las 6 horas (p:0.004).

Datos de isoniacida

Hubo diferencias significativas en la Cmax de isoniacida en el día de ayuno (4.54 mg/L) comparado con el día de no-ayuno (3.51 mg/L). (Wilcoxon Signed rank test p< 0.001) y en el AUC_{0-6} (13.31 mg·h/L *vs.* 12.11 mg·h/L; p: 0.001). Sin embargo no hubo diferencias en el género en cuanto a la Cmax o al $AUC_{0-6.}$

En el análisis multivariado (modelo de regresión multinivel), la Cmax de isoniacida fue un 14% mayor en el día de ayuno en comparación al día de no ayuno (p<0.001) y la dosis de isoniacida recibida también afectó a los niveles de isoniacida (p:0.038). Cuando la variable dependiente fue el AUC_{0-6}, el ayuno también incrementó los niveles de rifampicina un 22% (p<0.001). Un retraso en el Tmax (Tmax 6h) también disminuyó el AUC_{0-6} un 47% (p:0.013).

Score radiografía

El método diseñado para evaluar las radiografías, mostró una correlación inter-evaluador del 99.5% (0.991-0.997, p<0.001) para el Ralph score.

CONCLUSIONES

Las dosis de rifampicina e isoniacida deberían ajustarse por el peso del paciente. El primer estudio mostró un retraso en la absorción de la rifampicina y unos niveles bajos de rifampicina en comparación a los anteriormente reportados en la literatura como niveles terapéuticos independientemente de la co-morbilidad asociada. La farmacocinética de isoniacida se vio influenciada por la dosis recibida y aunque los niveles fueron bajos en un 30%, los niveles de AUC_{0-6} fueron similares a los de otros estudios. En el segundo estudio se ha demostrado, que la ingesta de la medicación con comida disminuye significativamente los niveles de Cmax y de AUC_{0-6} de rifampicina y de isoniacida. En ninguno de los estudios, la respuesta al tratamiento no se ha condicionado por una farmacocinética alterada ni de rifampicina ni de isoniacida. Otros factores como el sexo pueden afectar significativamente a la farmacocinética de rifampicina, pero no de isoniacida. La parasitosis intestinal no parece alterar la farmacocinética de rifampicina ni de isoniacida. Finalmente, el estado acetilador puede contribuir a una alteración de los niveles plasmáticos de isoniacida.

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ANNEXES

ANNEX 1

ARTICLES

ANNEX 2

STUDY QUESTIONNAIRES

DIET DIARIES

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Pharmacokinetics of Rifampin in Peruvian Tuberculosis Patients with and without Comorbid Diabetes or HIV

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For drug-compliant patients, poor responses to tuberculosis (TB) treatment might be attributable to subtherapeutic drug concentrations. An impaired absorption of rifampin was previously reported for patients with diabetes mellitus (DM) or HIV. The objectives of this study were to determine whether TB drug pharmacokinetics differed in Peruvian TB patients with DM or HIV. In this cross-sectional study, TB patients, recruited from health centers in Lima, Peru, had blood samples taken at 2 and 6 h after directly observed TB drug ingestion, to determine plasma concentrations of rifampin. Of 105 patients, 50 had TB without a comorbidity, 26 had coexistent DM, and 29 had coexistent HIV. Unexpectedly, the overall median 2- and 6-h levels of rifampin were 1.6 and 3.2 mg/liter, respectively, and the time to the peak concentration was 6 h (slow absorber) instead of 2 h (fast absorber) for 61 patients (62.2%). The geometric mean peak concentration of drug in serum (C_{max}) was significantly higher in fast absorbers than in slow absorbers (5.0 versus 3.8 mg/liter; $P = 0.05$). The rifampin C_{max} was significantly lower in male patients than in female patients (3.3 versus 6.3 mg/liter; $P < 0.001$). Neither slow nor fast absorbers with comorbidities (DM or HIV) had significantly different C_{max} results compared to those of TB patients without comorbidities. An analysis of variance regression analysis showed that female gender ($P < 0.001$) and the time to maximum concentration of drug in serum (T_{max}) at 2 h ($P = 0.012$) were independently correlated with increased exposure to rifampin. Most of this Peruvian study population exhibited rifampin pharmacokinetics different from those conventionally reported, with delayed absorption and low plasma concentrations, independent of the presence of an HIV or DM comorbidity.

Over the last few decades, noncommunicable diseases (NCDs) have exhibited an increasing trend in developing countries (4), and the disease burden falls predominantly in these regions in terms of health and economic impacts (14). Diabetes mellitus (DM) is one of the major NCDs, and global statistics estimate an increase from 194 million people with this disease in 2003 to 330 million in 2030 (42). A substantial proportion (75%) will be living in developing countries (32), where concurrently, many communicable diseases, such as tuberculosis (TB), remain highly prevalent, particularly in countries with high rates of HIV infection (40).

The increased risk of active TB in diabetic patients is now well recognized (2, 31), and the danger of the convergence of these two global pandemics is clear, with 8 of the 10 countries with the highest incidences of DM in the world also presenting a major burden of TB (41).

Although less prevalent than DM, HIV is a more potent risk factor for TB and is a major cause of morbidity and mortality in HIV patients in low-income countries (9).

Most TB patients treated with standardized-dosing drug regimens exhibit a high cure rate with few side effects (19). Poor compliance with treatment is the major cause of treatment failure and relapse. Nevertheless, recently reported *in vitro* and animal model data suggest that the pharmacokinetic variability of antituberculosis drugs might be another factor to be considered (35). Therefore, an unknown percentage of cases with a poor response to treatment (defined by clinical failure or relapse) may be attributable to low drug concentrations (6, 15, 22, 27, 36). Therapeutic drug monitoring (TDM) has been proposed as a potential approach for patients failing therapy despite appropriately executed directly observed therapy (DOT) (27). Although TB drug dosing is based upon patient weight banding, antituberculosis drug pharmacokinetics (PK) may be altered by several factors, including age, gender, ethnicity, drug formulations, drug interactions, and gastroenteritis (6). Impaired absorption was previously suggested to occur in some patients with DM, HIV/AIDS, or cystic fibrosis (7, 12, 23, 33).

Culture conversion may be delayed in treated TB patients with DM; the only (small) study to evaluate PK in diabetic TB patients suggested that serum drug concentrations may be lower than those in TB patients without DM (23). For patients with HIV, conflicting results have been reported (7, 8, 11, 25, 29, 33, 37), although it was proposed that impaired pharmacokinetics leading to low drug concentrations could contribute to acquired drug resistance in fully adherent patients (39). This operational research study was undertaken to determine whether TB drug PK differed in Peruvian TB patients with a comorbidity of DM or HIV after controlling for potential confounders such as body weight

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(and, thus, the dose received in mg/kg of body weight) and intestinal parasitic infection.

MATERIALS AND METHODS

Study design, participants, and setting. This cross-sectional observational study was conducted in Lima, Peru, from July to December 2009. TB patients who had received at least 15 days of treatment were recruited from health centers in Lima where directly observed therapy (6 days per week during the intensive phase and twice a week during the maintenance phase) is provided through the DOTS program of the Peruvian National Tuberculosis Program (NTP). Patients unwilling or unable to give informed consent were excluded. Patients with known HIV disease or DM were particularly sought, with the aim of recruiting at least 25 individuals for each subgroup.

Field methods. At the interview, a semistructured questionnaire was administered to all participants. Particular emphasis was put on detecting previous gastrointestinal surgery, ongoing chronic diarrhea (three or more unformed stools per day for >15 days), and other factors that could contribute to malabsorption. Gender, age, height (cm), and weight (kg) were recorded, and the body mass index (BMI) was calculated.

Blood was drawn at the health center by dedicated study staff into 10-ml lithium heparin tubes at two time points, 2 and 6 h, after directly observed TB drug ingestion, and a fecal sample was collected for parasitological analysis.

Laboratory methods. Blood samples were refrigerated, kept in the dark, and transported to the Universidad Peruana Cayetano Heredia (UPCH), where serum was separated (by centrifugation at 2,000 rpm for 10 min) between 2 and 5 h after sample collection. Aliquots were stored at −70°C until they were batched and transported to the pharmacokinetics laboratory of the Liverpool School of Tropical Medicine (LSTM), where rifampin (RIF) levels were measured by using a validated high-performance liquid chromatography (HPLC) technique. All sample preparations were carried out in a darkened room. Two hundred microliters of each sample was assayed alongside a plasma calibration curve (0 to 32 μg/ml) and quality control samples with a low concentration of 1.5 μg/ml, a medium concentration of 17 μg/ml, and a high concentration of 26 μg/ml. Plasma was precipitated by using 50 μl of an ethanol-containing internal standard (butyl 4-hydroxybenzoate at 16 μg/ml), followed by the addition of 1 ml of methanol, and vortexed for 20 s. Tubes were incubated at 4°C for 1 h and then centrifuged at 2,000 × *g* for 10 min. One milliliter of the upper solvent layer was transferred into a clean glass soda tube, evaporated to dryness under a stream of nitrogen at 30°C, and reconstituted in 120 μl methanol, and 100 μl of sample was then injected onto a Shimadzu LC 2010 HT HPLC system, with detection at 254 nm. Data acquisition was performed by using Chromeleon (Dionex). The compounds were separated on a Luna C_8 150- by 4.6-mm 5-μm column (Phenomenex Inc.) protected by a LiChrosphere Si 60 5-μm column (VWR) using a gradient system, with the mobile phase containing solvent A (35% acetonitrile and 65% 50 mM ammonium formate [pH 5], adjusted with formic acid) and solvent B (70% acetonitrile and 30% 50 mM ammonium formate [pH 5], adjusted with formic acid). The column temperature was set at 30°C.

The assay was linear ($r^2 > 0.99$) in the concentration range of 0 to 32 μg/ml, with intra- and interday precisions with a <13% coefficient of variation (CV) and a <11% CV, respectively. The lower limit of quantification (LLOQ) (0.5 μg/ml) has been accepted as the lowest point on the standard curve, with a relative standard deviation of less than 10% and a 5:1 signal-to-noise ratio. The determination of RIF stability following three freeze-thaw cycles showed that for all quality control (QC) samples, there was a <9% CV in RIF concentrations. RIF stability after heat inactivation showed a <11% CV in RIF concentrations.

Serum glucose levels were measured for all patients, and glycosylated hemoglobin levels (HbA1c) were measured in diabetic patients. Fecal samples were concentrated by the Formol ether method and examined for the presence of ova, cysts, and parasites within 24 h of collection.

Measurements of pharmacokinetic outcomes. Two- and six-hour plasma concentrations were determined, and for each patient, the maximum serum drug concentration (C_{max}) was estimated as the higher of the two measured concentrations; the T_{max} for each patient was the time point at which the C_{max} occurred.

C_{max} values were also categorized as normal (>8 mg/liter), low (4 to 8 mg/liter), or very low (<4 mg/liter), in accordance with data reported previously (28, 38).

Statistical analysis. Data were double entered into an EpiData database; checked with EpiData software, version 3.1; and analyzed with STATA, version 10.

Demographic and general characteristics of the three patient groups (TB, TB-HIV, and TB-DM) were compared by using the chi-square (χ^2) test for the comparison of proportions and the Student *t* test for continuous and normally distributed variables. Non-normally distributed data were analyzed by using the Mann-Whitney test. The primary comparison of interest was between-group serum rifampin levels at 2 and 6 h postdosing and were analyzed with the independent-sample *t* test on the natural-logarithm-transformed pharmacokinetic data. Patients with TB and no other comorbidity ("TB") were compared with (i) patients with TB and DM ("TB-DM") and (ii) patients with TB and HIV ("TB-HIV"). A stratified analysis was performed to assess the effects of gender, age, BMI, and the dose of rifampin administered. The dose of rifampin was calculated by dividing the total milligrams of rifampin received by the weight of patients in kg.

A multivariate analysis of variance was performed to assess the variation in rifampin pharmacokinetics (C_{max}) attributable to the presence of a comorbidity (HIV or DM), gender, BMI, and other variables that emerged from the univariate analyses.

Ethics and institutional review. The study protocol and consent form were approved by the ethics committee of the London School of Hygiene and Tropical Medicine (LSHTM), the institutional review boards of the UPCH and Dirección de Salud-II (DISA-II) Lima Sur (regional Ministry of Health), and the ethics committee of the Hospital Nacional Dos de Mayo, Lima, Peru.

RESULTS

Participant characteristics. Participant characteristics are shown in Table 1. Of 113 patients recruited into the study, PK data were available for 105 (mean age, 36.9 years; 63.8% male patients); for 5 of these patients, only one measurement was available, because either the sample was insufficient or the 6-h sampling time was missed. Sixty-one patients were in the intensive phase and 44 were in the continuation phase of their treatment; 70.5% of cases were new cases. The majority of patients (87%) had pulmonary TB; 77 had microbiologically confirmed TB, while 28 were receiving treatment based on clinicoepidemiological and radiological grounds. Fifty patients had TB without a comorbidity (TB, the baseline group for comparison), 26 had coexistent DM (TB-DM), and 29 had coexistent HIV (HIV-TB). Diabetic TB patients were significantly older and had significantly higher BMIs and significantly higher blood glucose levels than TB patients without a comorbidity ($P < 0.001$ for all). The median HbA1c level for diabetic patients was 8.3% (range, 4.6 to 12.8%; data were available for only 23/26 patients).

A significantly higher proportion of HIV-TB patients were male, but otherwise, this group did not differ significantly from TB patients without a comorbidity. The mean CD4 count, available for only 14 patients, was 298 cells/μl. Eight HIV patients were receiving antiretroviral therapy, none with protease inhibitors.

The dose of rifampin (provided by IQ-farma and Infarmasa-Corporación, both from Peru) recommended by the Peruvian NTP is 10 mg/kg/day to a maximum of 600 mg per day for both

TABLE 1 Participant characteristics by subgroup[a]

Parameter	Value for group			
	TB ($n = 50$)	DM-TB ($n = 26$)	HIV-TB ($n = 29$)	All ($n = 105$)
% male patients	48.0	65.4	90.0***	63.8
Mean age (yr) (range)	31.1 (18–65)	51.3 (29–79)***	34.1 (24–48)	36.9 (18–79)
No. of patients with microbiologically confirmed TB (%)	38 (76.0)	22 (84.6)	17 (58.6)	77 (73.3)
Mean BMI (kg/m^2) (range)	23.3 (18.4–29.6)	27.5*** (21.9–36.3)	22.8 (15.8–34.8)	24.2 (15.8–36.3)
No. of patients with diarrhea <15 days (%)	1 (2.0)	3 (11.5)	2 (6.9)	6 (5.7)
No. of patients with chronic diarrhea (%)	0 (0)	0 (0)	2 (6.9)	2 (1.9)
No. of patients with intestinal surgery (%)	4 (8.0)	4 (15.4)	2 (6.9)	10 (9.5)
Mean blood glucose level (mg/dl) (range)[b]	92.7 (40–159)	178.8 (85–441)***	92.7 (61–123)	114.2 (40–441)
No. of patients with pathogenic parasites (%)[c,d]	4 (8.0)	1 (3.9)	4 (14.8)	9 (8.7)
No. of patients with nonpathogenic parasites (%)[c]	16 (32.0)	12 (46.2)	7 (25.9)	35 (34.0)
No. of patients with intensive-phase treatment (%)	31 (62.0)	13 (50.0)	17 (58.6)	61 (58.1)
Median dose of rifampin received (mg/kg) (range)	10.1 (7.4–12.6)	8.8 (5.7–10.9)***	10.3 (6.7–15.8)	9.8 (5.7–15.8)

[a] Proportions are expressed as cases/total number of patients (percent). Numerical values are expressed as means (ranges). All the tests compared the DM-TB or HIV-TB group to the TB (non-HIV, non-DM) group. Continuous variables were analyzed by using an independent *t* test, and categorical variables were analyzed with a Pearson χ^2 test. BMI, body mass index. ***, $P < 0.001$, compared with the TB group without a comorbidity.

[b] Results were not available for one TB patient (no comorbidity), one TB-DM patient, and two TB-HIV patients.

[c] Results were not available for 2 HIV patients.

[d] Two HIV patients had asymptomatic giardiasis, 1 patient had *Cyclospora cayetanensis*, 1 patient had *Cryptosporidium parvum*, and 1 diabetic patient also had giardiasis; in the TB group, all 4 patients had giardiasis.

the intensive and maintenance phases. The calculated dosage received was significantly lower in the DM-TB group (Table 1) and was significantly lower overall in males (9.5 mg/kg; range, 8.3 to 10.5 mg/kg) than in females (10.5 mg/kg; range, 9.3 to 11.0 mg/kg) ($P = 0.004$ by a Welch *t* test).

Pharmacokinetic analysis. (i) Rate of rifampin absorption (T_{max}). Unexpectedly, the T_{max} was at 6 h instead of at 2 h for 61 patients (62.2%). For further analysis, subjects were therefore divided into 2 groups, (i) fast absorbers, with T_{max} at 2 h, and (ii) slow absorbers, with T_{max} at 6 h. Being a slow absorber (with a delayed T_{max}) was not associated with gender, age group, DM or HIV comorbidity, phase of treatment, intestinal parasitic infection, or dose received (data not shown).

(ii) Peak rifampin level (C_{max}). Data are expressed as geometric means since the analysis was performed with the natural logarithm of the C_{max}. Overall, median 2- and 6-h levels of rifampin were 1.6 mg/liter (range, 0 to 18 mg/liter) and 3.2 mg/liter (range, 0 to 16 mg/liter), respectively. The magnitude of the geometric mean peak rifampin absorption (C_{max}) was 4.2 mg/liter (range, 0.5 to 18 mg/liter). The geometric mean C_{max} of fast absorbers was significantly higher (5.0 mg/liter) than that of slow absorbers (3.8 mg/liter) ($P = 0.05$).

The rifampin C_{max} was significantly lower in male than in female patients (3.3 versus 6.3 mg/liter; $P < 0.001$), and this effect was consistent in both fast (4.4 versus 6.7 mg/liter; $P = 0.009$) and slow (2.7 versus 6.1 mg/liter; $P < 0.001$) absorbers (Fig. 1).

Neither slow nor fast absorbers with comorbidities had results that were significantly different C_{max} from those of TB patients

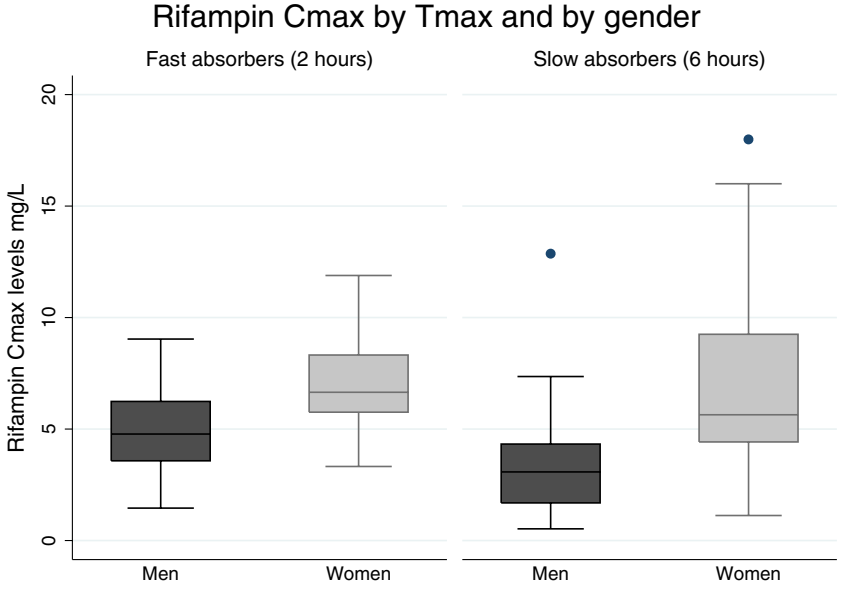

FIG 1 The rifampin C_{max} is lower in males regardless of the T_{max}.

TABLE 2 Determinants of C_{max} in fast and slow absorbers of rifampin[a]

Determinant	Geometric mean C_{max} (mg/liter) for fast absorbers (2 h) ($n = 37$)	*P* value for fast absorbers	Geometric mean C_{max} (mg/liter) for slow absorbers (6 h) ($n = 61$)	*P* value for slow absorbers
TB comorbidity				
DM-TB	5.1	0.99	3.4	0.29
HIV-TB	4.8	0.76	3.1	0.10
None	5.1		4.5	
Sex				
Male	4.4	0.009	2.7	<0.001
Female	6.7		6.1	
Phase of treatment				
Intensive phase	5.5	0.64	3.4	0.06
Maintenance phase	4.9		4.7	
Age group				
18–30 yr	5.0	0.93	4.4	0.61
31–40 yr	4.9		3.5	
>40 yrs	5.2		3.5	
Presence of intestinal parasites				
Yes	3.9	0.40	3.0	0.49
No	5.1		3.8	
Overall geometric mean	5.0		3.8	

[a] Numerical values are expressed as geometric means. Comparisons of groups were made between DM-TB or HIV-TB group and the TB (non-HIV, non-DM) group. All the variables were analyzed by using the independent-sample *t* test on the natural logarithm-transformed data.

without comorbidities (Table 2). The rifampin dosage received had no discernible effect upon C_{max} values among either slow or fast absorbers.

Thirty-six patients (34.3%) had undetectable rifampin levels at 2 h, two of whom also had undetectable levels at 6 h (Fig. 2). In the fast-absorber group, 10 patients (27.0%) had C_{max} values of <4 mg/liter, 23 (62.2%) had rifampin levels between 4 and 8 mg/liter, and only 4 (10.8%) had values that are regarded as acceptable normal levels. For slow absorbers, 31 (50.8%) had C_{max} values of <4 mg/liter, 20 (32.8%) had levels between 4 and 8 mg/liter, and 10 patients (16.3%) had normal levels (>8 mg/liter) (Fig. 2).

A multivariate analysis was performed, where the dependent

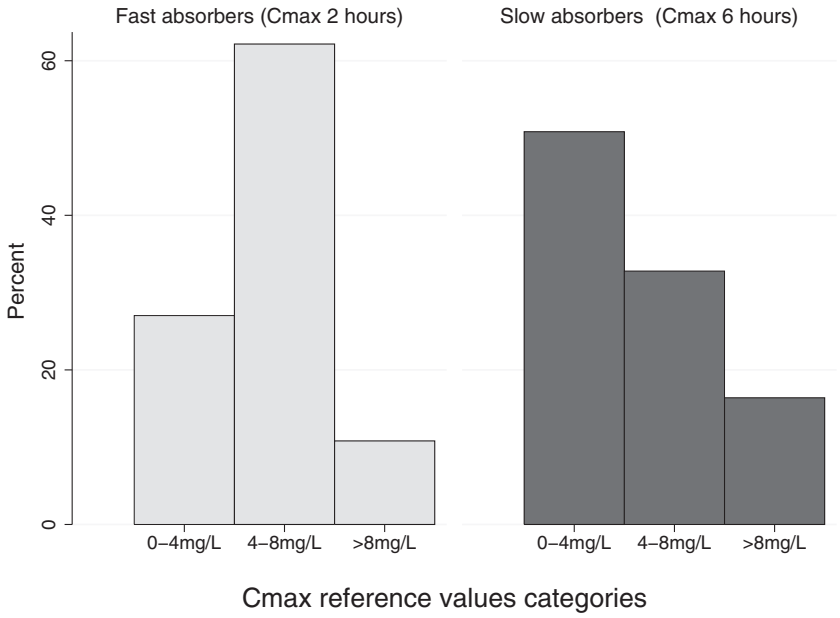

FIG 2 Frequency distribution of C_{max} categories by slow- and fast-absorber subgroups.

TABLE 3 Multivariate regression model of the independent association of various variables with exposure to rifampin[a]

Variable	Proportional difference	CI	*P* value[d]
T_{max} (6 h)	0.71[b]	0.55–0.93	**0.012**
Sex (female)	1.91[c]	1.42–2.56	**<0.001**
Rifampin dose received	1.05	0.96–1.14	0.299
Comorbidity			
HIV	0.98	0.71–1–37	0.926
DM	0.99	0.71–1.37	0.931

[a] The model was considered based on the natural logarithm of the C_{max} values. The proportional difference was calculated as the exponential of the coefficient obtained for each variable in the multivariate model.
[b] For interpretations of the proportional difference, C_{max} levels in slow absorbers were 29% lower than C_{max} levels in fast absorbers.
[c] For interpretations of the proportional difference, C_{max} levels in women were 91% higher than those in men.
[d] Boldface indicates significant difference.

variable was "the natural logarithm of C_{max}." We built a model to assess the independent effect of the time of absorption, gender, comorbidity (DM or HIV associated with TB), and rifampin dose received. The rifampin dose received was not found to have an effect upon the C_{max}. However, males were found to receive lower doses than females. Therefore, it was decided to include the variable rifampin dose received in the multivariate analysis. Other variables, such as age, serum glucose level, or the presence of intestinal parasites, were not significantly associated and were excluded during the building of the model (Table 3). The rifampin dose received did not significantly influence the C_{max} in our model. However, males were found to have C_{max} levels 1.9 times lower than those of females (confidence interval [CI], 1.42 to 2.56; *P* value, <0.001), and fast absorbers also differed significantly compared to slow absorbers (0.71; CI, 0.54 to 0.93; *P* value, 0.012) in our model.

Since (unexpectedly) many patients had undetectable levels at 2 h and many others had rifampin levels that were higher at 6 h than at 2 h, the area under the curve could not be calculated.

It is recommended that rifampin be taken on an empty stomach, as a modest reduction in absorption was previously observed when rifampin was taken with food. Information on oral intake was available for only 48 patients, 12 of whom fasted for at least 2 h before taking the treatment. In this small, underpowered subgroup analysis, oral intake was not associated with a delayed T_{max} or with a reduced C_{max}. Two Peruvian companies provide rifampin to the NTP. Health centers do not necessarily receive only a single brand, and it was possible to reliably identify the manufacturer for only 48 patients. Thirty-eight patients (80.9%) had been treated with drugs from manufacturer "A," while 9 (19.2%) had been treated with drugs produced by manufacturer "B." The median plasma rifampin C_{max} values achieved did not differ between manufacturers (4.5 mg/liter for manufacturer A [range, 0 to 15.6 mg/liter] versus 4.9 mg/liter [range, 1.5 to 12.9 mg/liter] for manufacturer B; $P = 0.7$).

The treatment responses of 99 out of the 105 TB patients were evaluated 6 months after the completion of treatment. Ten patients were found to have multidrug-resistant TB (MDR-TB) and changed treatment schema after the PK analysis and were therefore not included in this subanalysis. A further 29 patients either had abandoned treatment and restarted several months later or were lost to follow-up. Of the remaining evaluable 60 patients, 55 were considered completely cured and relapse free 6 months after finishing treatment, and 5 had an unfavorable outcome. Out of these 5 patients, 2 of them were diabetic patients, 2 were HIV positive (1 of the HIV-positive patients had extrapulmonary cerebral TB without improvement despite prolonged therapy), and 1 patient had no comorbidity. Four of these 5 patients had an absorption delay (T_{max} at 6 h instead of at 2 h), 3 of them had very low C_{max} levels, and the other 2 had low levels (4 to 8 mg/liter). Among the 55 patients with a good outcome, 31 patients (56%) had delayed absorption, 18 patients (33%) had C_{max} levels of <4 mg/liter, 26 (47%) had low levels, and 8 (15%) had normal C_{max} levels. For 3 of these patients with a good outcome, neither the T_{max} nor the C_{max} could be calculated because only 1 blood sample was available.

DISCUSSION

The key finding of this research study, carried out under real-life field conditions and not in the controlled environment of a dedicated PK unit, is that measured serum rifampin levels in patients receiving directly observed TB treatment in the Peruvian NTP were highly variable and very frequently well below what is conventionally regarded as a therapeutic range. This study was designed to determine to what extent a DM or HIV comorbidity might impair rifampin pharmacokinetics, but neither of these conditions had any demonstrable effect on either the rate of rifampin absorption (T_{max}) or the magnitude of absorption (C_{max}). Instead, there was a widespread perturbation of what is considered to be "normal" rifampin pharmacokinetics across all patient groups. Specifically, two-thirds of patients had delayed rifampin absorption, with higher levels at 6 h than at 2 h postdosing, and for only one-quarter of patients was a measured rifampin serum level (whether at 2 or 6 h) above the 8-mg/liter threshold regarded as necessary for therapeutic efficacy.

Although therapeutic drug monitoring (TDM) is neither widely used nor recommended during TB treatment, it is widely held that abnormal TB drug pharmacokinetics may adversely influence outcomes, with potential consequences including treatment failure, relapse or death, prolonged infectiousness, and the development of acquired drug resistance (5, 39). In addition, TDM might contribute not only to the identification patients with low levels of rifampin but also to a shrinking of the duration of treatment (13), since there are many patients in directly observed therapy (DOT) programs who complete the treatment in 1 year rather than in 6 months.

We observed very low levels of rifampin at 2 h in 68.6% of patients, which is in accordance with data from other studies (3, 38). Moreover, 36 patients (34.3%) had undetectable levels at 2 h, when the peak concentration after oral administration theoretically occurs (23, 26). Wilkins et al. previously reported considerable interindividual variability in rifampin pharmacokinetics in South Africa and suggested that highly variable rates of absorption could significantly impact the C_{max}, since, as further confirmed by our data here, slower absorption leads to lower peak plasma concentrations (43). In our study, 61 patients had higher levels of rifampin at 6 h than at 2 h, which we attributed to a delay in absorption; thus, the T_{max} varied significantly. Aside from interindividual variability, we wanted to explore other potential explanations for our startling findings. A strength of this study was its real-life execution in health centers; thus, samples were taken

from patients doing what they do every day and not under the tightly controlled conditions of a dedicated PK ward. As such, despite the known effect of food upon the pharmacokinetics of rifampin (and other TB drugs) (5, 34, 44), many patients did not fast before taking treatment; indeed, fasting is not a feature of the Peruvian NTP guidelines. In the small subgroup of patients for whom oral intake data were available (48 of 105), we could not demonstrate any effect of food on the C_{max} or T_{max}.

Men received a lower mg/kg dosage and had lower maximum rifampin levels than women. However, even after adjusting for the rifampin dosage received in the multivariate analysis, men had significantly lower plasma levels than women, a finding reported previously (21, 38) and likely due to an increased volume of distribution (24).

No association was found between rifampin PK and intestinal parasitosis, which affected almost 10% of participants, which does not support the hypothesis that intestinal parasitosis might affect TB treatment outcomes through a PK-related mechanism (18).

The absorption of orally administered drugs can be modified by the nonspecific adsorption of drugs to excipients in formulations (5); a study in South Africa documented a poor relative bioavailability of rifampin from some fixed-dose combinations (30). It was not clear whether bioequivalence studies had been performed for the two generic preparations of rifampin in use (10); although data were incomplete, we were unable to demonstrate a difference in the PK results for each preparation.

If DM is confirmed to be an independent risk factor for treatment failure (1), our data suggest that this is not related to deranged pharmacokinetics of rifampin, nor is it associated with hyperglycemia, although the limited pharmacokinetic analysis might hamper the assessment of the effect of DM on the pharmacokinetics of rifampin. It remains controversial whether a significant malabsorption of antimycobacterial drugs occurs in HIV patients (9, 11, 29, 33, 37) and, if so, whether this is associated with treatment failure or the emergence of drug resistance. Although a crude analysis revealed marginally reduced C_{max} values in HIV patients (data not shown), after stratifying data by the T_{max}, no associations remained between the rifampin level and HIV status in either fast or slow absorbers. HIV-related achlorhydria, HIV enteropathy (17), and opportunistic infections of the intestinal tract were reported previously (5, 16) to be risk factors for a poor treatment outcome; we did not explore these risk factors other than in the search for intestinal parasites.

The main limitation of this work was the inability to calculate the 0- to 6-h area under the PK curve (AUC). We sampled only two time points, 2 and 6 h, for pragmatic and logistic reasons and because this approach was previously successful. However, the real C_{max} might have occurred between the 2- and 6-h samplings, and furthermore, the unexpected finding of so many measurements below the limit of detection and the fact that a considerable proportion of patients had their T_{max} at 6 h rendered AUC calculations futile. While we regarded the conduct of the study under real-life circumstances as being important and a strength of the data, we were unable to adequately control and reliably measure concomitant oral intake for all participants. Gastrointestinal upset is common in TB patients, and many gain relief by taking medicines with food or juice; a future study might usefully examine detailed PK curves for the same patient on consecutive days under different conditions of oral intake. A perennial and inherent problem with TB drug PK analyses is unpicking whether plasma rifampin levels, defined as the C_{max}, or (preferentially) total exposure, measured as the AUC, is the crucial determinant of drug efficacy at the cellular level and, if so, what the minimum drug exposure (measured in plasma) needs to be in order to have a high probability of efficacy. Evidence for poorer treatment outcomes associated with impaired pharmacokinetics is, perhaps surprisingly, somewhat thin and less persuasively demonstrated for rifampin (38) than for pyrazinamide (6). Moreover, treatment outcomes of patients in Peru with standardized regimens using these same drugs and dosages are generally good, implying that efficacy, at least with the existing four-drug combination therapy, is adequate. We could evaluate treatment outcome and relapse rates only to 6 months for 60 patients, 5 of whom had an unfavorable outcome. Given the very low levels of rifampin among patients cured of disease, further investigation is needed to ascertain whether a review of the purported normal C_{max} level of >8 mg/liter is warranted. However, some studies have demonstrated that an increase in the dose of rifampin led to a better treatment outcome (20). Therefore, if we assume a higher median concentration in those patients who received a higher dose, we could consider that there is a correlation between the concentration and the treatment outcome.

In addition, we report data only on rifampin here, and patients might have been cured despite impaired rifampin pharmacokinetics because of the effect of the combination therapy (isoniazid, ethambutol, or pyrazinamide). For this reason, the 8-mg/liter threshold might be better regarded as desirable rather than necessary.

We have demonstrated markedly deranged rifampin pharmacokinetics in a majority of TB patients taking directly observed therapy in the Peruvian NTP, with delayed absorption and low drug levels, findings not inconsistent with those of previous work in other countries. A clarification of the implications of these findings for clinical outcomes is now essential; if outcomes are unaffected, perhaps further thought needs to be given to our existing paradigm for normal, therapeutically active rifampin pharmacokinetics.

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Effects of Dosage, Comorbidities, and Food on Isoniazid Pharmacokinetics in Peruvian Tuberculosis Patients

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Poor response to tuberculosis (TB) therapy might be attributable to subtherapeutic levels in drug-compliant patients. Pharmacokinetic (PK) parameters can be affected by several factors, such as comorbidities or the interaction of TB drugs with food. This study aimed to determine the PK of isoniazid (INH) in a Peruvian TB population under observed daily and twice-weekly (i.e., biweekly) therapy. Isoniazid levels were analyzed at 2 and 6 h after drug intake using liquid chromatography mass spectrometric methods. A total of 107 recruited patients had available PK data; of these 107 patients, 42.1% received biweekly isoniazid. The mean biweekly dose (12.8 mg/kg of body weight/day) was significantly lower than the nominal dose of 15 mg/kg/day ($P < 0.001$), and this effect was particularly marked in patients with concurrent diabetes and in males. The median maximum plasma concentration (C_{max}) and area under the concentration-time curve from 0 to 6 h (AUC_{0-6}) were 2.77 mg/liter and 9.71 mg·h/liter, respectively, for daily administration and 8.74 mg/liter and 37.8 mg·h/liter, respectively, for biweekly administration. There were no differences in the C_{max} with respect to gender, diabetes mellitus (DM) status, or HIV status. Food was weakly associated with lower levels of isoniazid during the continuation phase. Overall, 34% of patients during the intensive phase and 33.3% during the continuation phase did not reach the C_{max} reference value. However, low levels of INH were not associated with poorer clinical outcomes. In our population, INH exposure was affected by weight-adjusted dose and by food, but comorbidities did not indicate any effect on PK. We were unable to demonstrate a clear relationship between the C_{max} and treatment outcome in this data set. Twice-weekly weight-adjusted dosing of INH appears to be quite robust with respect to important potentially influential patient factors under program conditions.

Despite recent progress, global tuberculosis (TB) control is hampered by the impact of HIV coinfection (1, 2) and diabetes mellitus (DM) (3, 4) on the incidence of disease and treatment outcomes worldwide. Standardized short-course treatment regimens which are currently recommended by the WHO (5) remain the cornerstone of control strategies and may help to achieve high cure rates, although there is a need for proper evaluation of dosing schedules and of the optimal duration of treatment (6).

Several factors, including poor adherence (7), high bacillary burden (8), radiological cavitation (9), and DM (3), are associated with poor outcomes. HIV-positive people may be at risk of poorer outcomes during fully intermittent therapy without antiretroviral treatment (2) and remain at considerable risk of reinfection after successful treatment (10).

Debate continues about the impact of antituberculosis drug pharmacokinetics (PK) on clinical outcomes (11–15). Experimental and clinical studies have pointed to the influence of interpatient variability in key PK parameters, such as plasma area under the curve (AUC), on important outcomes, including treatment success and the emergence of resistance (16–19). These PK parameters may be affected by several factors, including age, gender, ethnicity, genetics, nutritional status, drug formulation and quality, and drug-drug interactions (20). Although data have accumulated on the impact of HIV on the PK of anti-TB drugs (11, 14), only a few studies have specifically examined the effect of DM, and their results have conflicted (21, 22).

Isoniazid (INH), one of the key agents in first-line TB therapy, has high early bactericidal activity (EBA) and exhibits prolonged postantibiotic effects (PAE) *in vitro*, which may be important for preventing the emergence of resistance during therapy (23). Its metabolism is controlled by the polymorphic *N*-acetyltransferase-2 (NAT2) locus, which results in relatively high interindividual variability in isoniazid PK (24). The maximum serum drug concentration (C_{max}) of INH generally occurs between 1 and 2 h after drug intake, although food, particularly high-fat meals, may delay and reduce overall absorption.

Although INH is theoretically well suited to intermittent administration, there are few modern data on the PK of the drug during intermittent dosing (25, 26), particularly twice-weekly regimens, which are used in several Latin American countries. While this approach is effective (6) and may help to promote adherence, its PK robustness in the face of important patient factors, such as DM, HIV, acetylator phenotype, and food effect, has not been extensively studied. We report here the results of a field PK study

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in Lima, Peru, which aimed to evaluate plasma concentrations of isoniazid under daily and twice-weekly dosing in these important patient subgroups.

MATERIALS AND METHODS

Field methods. This study was conducted in Lima, Peru, from July to December 2009. TB patients who were receiving directly observed first-line TB therapy through the national TB program and who had completed at least 15 days of treatment were invited to participate and provided written informed consent. Isoniazid in use in the Peruvian National Tuberculosis Program (PNTP) at the time of the study was sourced from IQ-Farma and the LCG corporation, both Peruvian companies. The PNTP recommends a daily dose of 5 mg/kg of body weight/day during the intensive phase of treatment and a biweekly dose of 15 mg/kg/day (maximum dose of 800 mg) during the continuation phase. Within the PNTP, TB drugs are not available as fixed-dose combinations. Patients with known HIV disease or DM were particularly sought, with the aim of recruiting at least 25 individuals for each subgroup. Blood sampling was performed 2 and 6 h after observed dosing under field conditions. In particular, intake of food and water was not controlled by the investigators. Patients were contacted again at the end of therapy and 6 months after treatment completion in order to evaluate the final treatment outcomes. This study was approved by the ethics committees of the London School of Hygiene and Tropical Medicine, Hospital Nacional Dos de Mayo, and Universidad Peruana Cayetano Heredia (UPCH) in Lima and the institutional review board of Dirección de Salud (DISA II) in Lima Sur (regional Ministry of Health).

Laboratory methods. Whole blood (10 ml) was drawn into lithium heparin tubes, kept in the dark, and transferred under refrigeration within 3 to 5 h to the research laboratory at UPCH. The samples were centrifuged at 2,000 rpm for 10 min, and plasma supernatant was removed and stored at −70°C prior to bioanalysis at the Liverpool School of Tropical Medicine. A total of 100 μl of plasma from each sample was assayed alongside a plasma calibration curve (range, 0 to 6,000 ng/ml) and quality control samples at low (40 ng/ml), medium (2,500 ng/ml), and high (5,000 ng/ml) concentrations of INH. Samples which tested above our highest level of quantification were diluted 1:4 and were reanalyzed with a fresh calibration curve and quality control samples.

Each sample underwent protein precipitation with 900 μl of an internal standard (IS) (acetonitrile containing 200 ng/ml of metformin). All the IS responses for the analytical runs carried out in our samples were <15% relative standard deviation, according to the bioanalytical guidelines approved by the FDA. The samples were vortexed for 20 s and were centrifuged at 14,000 × *g* for 20 min; 800 μl of supernatant was transferred to clean 5-ml soda-glass tubes and evaporated to dryness under nitrogen at 30°C. Dried-down samples were reconstituted in 100 μl of mobile phase (90% water, 10% methanol, and 0.3% formic acid) and were vortexed for 10 s. Reconstituted samples were transferred to insert vials and centrifuged at 4,700 × *g* for 5 min; 20 μl of the reconstitute underwent chromatographic separation on a Hypersil GOLD C_{18} column (150 by 4.6 mm, 3-μm particle size) (Thermo Scientific, Hemel Hempstead, United Kingdom) at 30°C using an isocratic gradient of 90% water, 10% methanol, and 0.3% formic acid at a rate of 300 μl/min. The high-performance liquid chromatography system was interfaced with a triple-quadrupole TSQ Quantum Access mass spectrometer (Thermo Scientific) with an atmospheric pressure chemical ionization (APCI) source. An E2M30 rotary vacuum pump (Edwards High Vacuum International, West Sussex, United Kingdom), an NM30LA nitrogen generator (Peak Scientific, Renfrewshire, United Kingdom), and 99% pure argon gas (10 liters) (BIP10; Air Products, Liverpool, United Kingdom) were used.

The mass spectrometer was operated in positive selective reaction monitoring (SRM) mode using transitions of *m/z* 138.2 to 121.1 for INH and 130.2 to 60.4 for IS, an optimized collision energy of 17 eV for INH and IS, a narrow scan width (0.1 *m/z*) and scan time (0.1 s) for all transitions, and the data collection system operating in centroid mode. The sheath and auxiliary gas flows (nitrogen gas) were 15 and 20 lb/in^2, respectively. The capillary temperature within the ion source was maintained at 250°C, the discharge current was set to 5 μA, the spray voltage was set to 4.5 kV, and the collision pressure was 1.5 mTorr (argon). All standard curves were adequately described using an equal-weighted linear regression equation for INH using the data acquisition software LCquan version 2.5.6 (Thermo Scientific, Hemel Hempstead, United Kingdom). The correlation coefficient (r^2) for all INH calibration curves exceeded 0.99. The lower limit of quantification (LLOQ) (10 ng/ml for INH) was accepted as the lowest point on the standard curve, with a signal-to-noise ratio of 5:1 and a coefficient of variation (CV) of <11% for INH; the CV ranged from 2% to 11% at all other calibration levels for INH. The determination of INH stability following three freeze-thaw cycles showed that all quality control samples were within a CV of 11% for INH concentrations.

Data analysis. For each patient, the C_{max} was defined as the higher of the two concentrations measured at 2 and 6 h, and the T_{max} was the time point at which the C_{max} occurred. PK parameters were obtained by noncompartmental analysis using the trapezoidal rule and the linear-up-log-down method. MIC data were not available, and no additional analysis of PK-pharmacodynamic (PD) parameters was developed. Although an internationally agreed-upon guideline for therapeutic drug monitoring is lacking, a normal INH C_{max} may be defined, by comparison with existing pharmacokinetic data, as 3 to 5 mg/liter after a 5-mg/kg daily dose and as 9 to 15 mg/liter after a biweekly dose of 15 mg/kg/day (20). A C_{max} level of <2 mg/liter after a 300-mg daily dose or a C_{max} level of <7 mg/liter after a 900-mg biweekly dose is regarded as inadequate and is an indication for dose adjustment, according to some experts (19). We categorized our PK data accordingly. For 5-mg/kg/day daily dosing, very low C_{max} levels were <2 mg/liter, low levels were 2 to 3 mg/liter, and normal levels were >3 mg/liter; for 15-mg/kg/day biweekly dosing, very low levels were <7 mg/liter, low levels were 7 to 9 mg/liter, and normal levels were >9 mg/liter. The chi-square test was used for the comparison of proportions, and the Student *t* test or Wilcoxon rank-sum test was used for continuous variables, depending on variable distribution. The data were analyzed with Stata (Stata Corp, College Station, Texas, USA) and R version 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The general characteristics of the patients are described in Table 1. The PK data were not available for 6 out of 113 patients initially recruited due to inefficient processing of the sample or difficulties with venipuncture. In 4 of the remaining 107 patients, the 6-h sampling time was missed. Overall, 62 patients (57.9%) were sampled while receiving treatment in the intensive phase, and 45 were sampled in the continuation phase. As previously reported for this population (27), the patients with DM were significantly older and had a significantly higher body mass index (BMI) than the TB patients without DM ($P < 0.001$ for both).

The overall mean dose received during the intensive daily dosing phase was 5.15 mg/kg/day. Because of their higher BMI, the patients with DM received a lower daily dose of isoniazid than the patients with TB only (4.47 versus 5.3 mg/kg, respectively; $P = 0.02$, *t* test) (Fig. 1). During the continuation phase, the mean biweekly dose received on the treatment days was 12.8 mg/kg/day, which was significantly lower than the recommended dose of 15 mg/kg/day ($P < 0.001$); and 89% of the patients received doses lower than the nominal dosage. This effect was particularly marked in the TB-DM group (11.6 versus 13.3 mg/kg/day, respectively; $P = 0.015$) (Fig. 1). The mean maintenance phase dose of isoniazid received was lower in males than in females (12.22 versus 13.48 mg/kg/day, respectively; $P = 0.008$).

The T_{max} occurred at 2 h in 86.4% of intensive phase patients and in 90.7% of continuation phase patients. In the intensive

TABLE 1 Participant characteristics by subgroup

Characteristic	TB ($n = 52$)[a]	DM-TB ($n = 25$)[a]	*P*	HIV-TB ($n = 30$)[a]	*P*	Total ($n = 107$)[a]
Sex (male)	26 (50)	16 (64)	0.2	26 (86.7)	**0.001**[b]	67 (63.81)
Age (yr)	29 (22.5–36)	50 (44–58)	**<0.001**	35 (30–38)	0.3	35 (26–44)
Confirmed TB[c]	40 (76.9)	21 (84)	0.5	17 (56.7)	0.055	78 (72.9)
BMI (kg/m^2)[d]	22.9 (21.23–24.89)	27 (24.9–29.8)	**<0.001**	22 (20.8–24.6)	0.5	23.7 (21.5–25.8)
Chronic diarrhea[e]	0 (0)	0 (0)		2 (6.7)	0.059	2 (1.9)
Intestinal surgery[f]	4 (7.7)	4 (16)	0.3	2 (6.7)	0.9	10 (9.35)
Blood glucose level (mg/dl)	95[g] (80–102)	119.5[c] (111–211.5)	**<0.001**	92.5[g] (81–102.5)	0.8	98[c] (84–114)
Pathogenic parasites	4 (7.7)	1 (4)	0.5	4[h] (14.3)	0.3	9[e] (8.6)
Nonpathogenic parasites	16 (30.8)	12 (48)	0.1	8[h] (28.6)	0.8	36[e] (34.3)
Intensive phase treatment	33 (63.5)	13 (52)	0.2	17 (56.7)	0.5	62 (57.9)

[a] Values shown are the number of cases (% of total) or median (interquartile range). All the tests compare the DM-TB or HIV-TB groups with the TB-only (non-HIV non-DM) group. Continues variables were analyzed with the independent *t* test, and categorical variables were analyzed with the Pearson chi-square test.
[b] Bold type indicates a significant difference.
[c] Confirmed TB indicates microbiologically confirmed TB cases.
[d] BMI, body mass index.
[e] Chronic diarrhea was defined as persistence of liquid depositions for >15 days.
[f] Intestinal surgery was surgery related to a gastrointestinal tube.
[g] For two HIV patients, one DM patient, and two TB control patients, we were unable to test the blood glucose level.
[h] For two HIV patients, feces samples were not processed.

phase patients, the median isoniazid concentration was 2.55 mg/liter (interquartile range [IQR], 1.67 to 3.5 mg/liter) at 2 h and was 0.59 mg/liter (IQR, 0.3 to 2.31 mg/liter) at 6 h. The median C_{max} was 2.77 mg/liter (IQR, 1.75 to 4.67 mg/liter). In continuation phase patients, the median concentrations of isoniazid at 2 and 6 h were 8.71 mg/liter (IQR, 4.01 to 23.27 mg/liter) and 3.1 mg/liter (IQR, 1 to 5.81 mg/liter), respectively. The median C_{max} was 8.74 mg/liter (IQR, 4.21 to 23.03 mg/liter). The percentage of patients in the intensive phase group that did not reach a reference value at 2 h after drug intake of at least 2 mg/liter (i.e., very low levels) was 34%; 33.3% of patients taking isoniazid biweekly did not achieve a reference value of 7 mg/liter at 2 h. The median AUC from 0 to 6

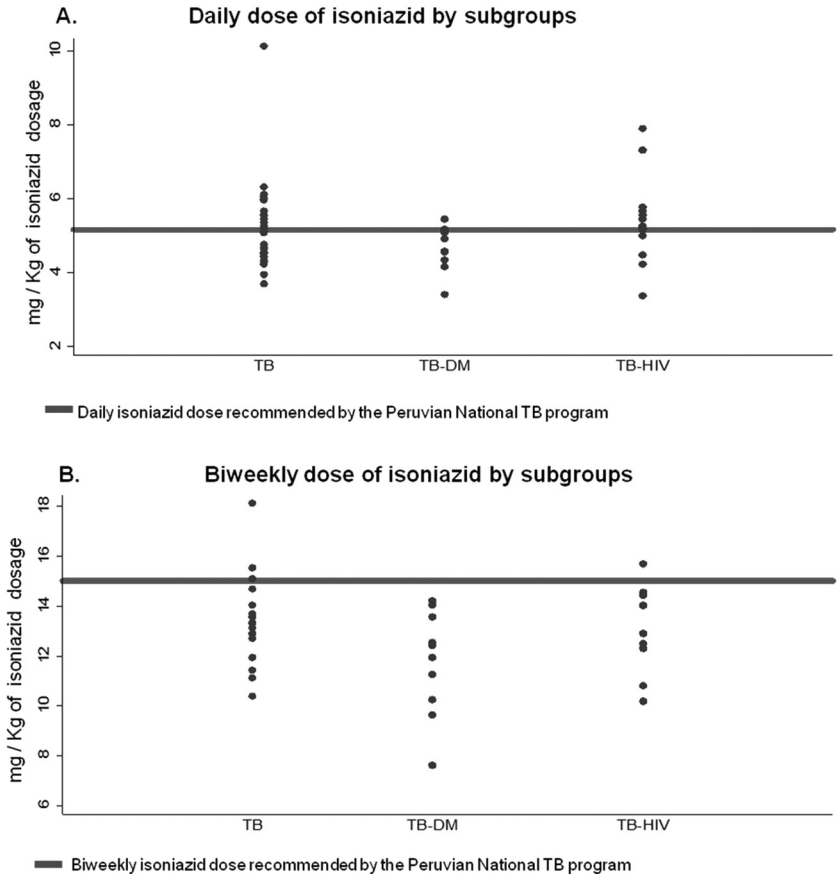

FIG 1 Daily (A) and biweekly (B) isoniazid doses, as recommended by the Peruvian National TB program.

TABLE 2 Determinants of the INH C_{max} and AUC_{0-6} in daily and twice-weekly doses

Determinant	Daily dose of INH						Twice-weekly dose of INH					
	n	Median C_{max} (mg/liter)	*P* value	n^b	AUC_{0-6} (mg·h/liter)	*P* value	*n*	Median C_{max} (mg/liter)	*P* value	n^b	AUC_{0-6} (mg·h/liter)	*P* value
TB comorbidity[a]												
DM	11	2.6	1	11	10.39	0.6	13	4.4	0.3	11	54.26	0.2
HIV	16	2.6	0.8	13	9.72	0.6	13	9.9	1	12	53.64	0.6
TB alone	32	3		27	11.05		18	8.6		16	62.45	
Sex												
Male	36	2.8	1	31	10.23	0.9	29	8.6	0.2	26	46.28	0.2
Female	23	2.8		20	11.1		15	21.4		13	79.71	
Fasting status with therapy												
Fasting	6	3.1	0.4	6	11.06	0.2	6	16.6	1	4	71.52	1
Nonfasting	25	2.1		20	6.96		9	7.7		8	53.17	
Age group												
18–30 yrs	22	3	0.8	20	10.61	0.6	17	9.9	0.9	15	40.51	0.7
31–40 yrs	23	2.5		18	8.77		12	7.4		10	31.45	
>40 yrs	14	2.9		13	9.71		15	8.8		14	28.32	
Intestinal parasites												
Yes	4	2.1	0.6	3	4	0.3	5	9	0.9	5	34.81	0.9
No	53	3		46	10.12		39	8.6		34	38.65	
Bacteriologically confirmed TB												
Yes	44	2.8	0.6	36	9.6	1	31	9.9	0.9	26	38.9	0.6
No	15	2.5		15	9.7		13	8.6		13	19.6	
Overall median	59	2.8		51	9.71		44	8.7		39	37.81	

[a] The TB comorbidity group compared the DM-TB or HIV-TB group with the TB-alone (non-HIV non-DM) group. All the variables were analyzed with the nonparametric Wilcoxon test, and the Kruskal-Wallis test was used for the age group variable.

[b] The numbers related to the C_{max} are slightly different than the numbers related to the AUC, because we were unable to calculate the AUC in cases of undetectable levels or delayed absorption (according to the C_{max} at 6 h).

h (AUC_{0-6}) was 9.71 mg·h/liter (5.87 to 13.31 mg·h/liter) for intensive phase patients and 37.8 mg·h/liter (19.2 to 82.26 mg·h/liter) for continuation phase patients. Since information about the NAT2 genotypes of participants was not available, a bivariate normal mixture model was fitted to the distribution of the half-lives to estimate the proportion of acetylator phenotypes from the data. Mixtures of normal distributions were fitted to PK parameter distributions using maximum likelihood in R. The best model for the distribution was bimodal, in which 53% of the population was estimated to be fast/intermediate acetylators with a mean half-life of 1.48 h, and 47% were estimated to be slow or intermediate acetylators with a mean half-life of 5.25 h.

The univariate analysis of factors influencing PK parameters is presented in Table 2. The relationship between the weight-adjusted dose and the PK exposure as measured by the AUC_{0-6} was demonstrated to be linear across the wide range of doses studied (Fig. 2). Information on food intake of the patients was available for only 48 patients, 12 of whom fasted for at least 2 h before taking the drugs, as recommended. Although the C_{max} and AUC_{0-6} appeared reduced in nonfasting patients, this was not a statistically significant finding (Table 2). Notably, none of the six patients in the intensive phase group who fasted before drug intake had 2-h concentration levels of <2 mg/liter, compared to 12/25 (48%) of intensive phase patients who did not fast ($P = 0.04$, Fisher' exact test), although this was not true in the maintenance phase group (33% fasting versus 22.2% nonfasting patients had 2-h concentration levels of <7 mg/liter; $P = 0.4$). In the univariate analysis, the C_{max} and AUC_{0-6} were unaffected by age, gender, DM, or HIV in either the intensive or the continuation phase (Table 2; Fig. 3). Multivariate models with log-transformed PK parameters as de-

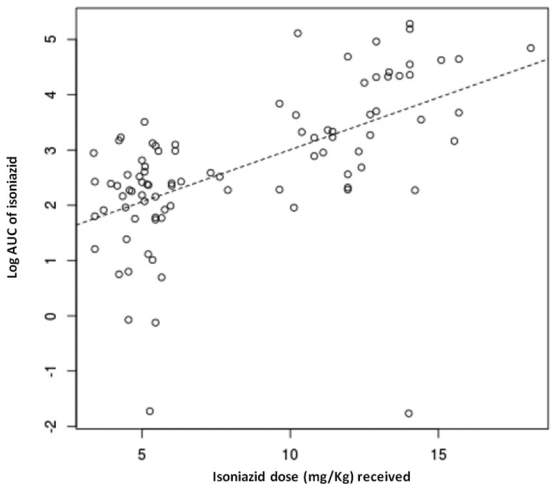

FIG 2 Relationship between dosage and exposure of isoniazid.

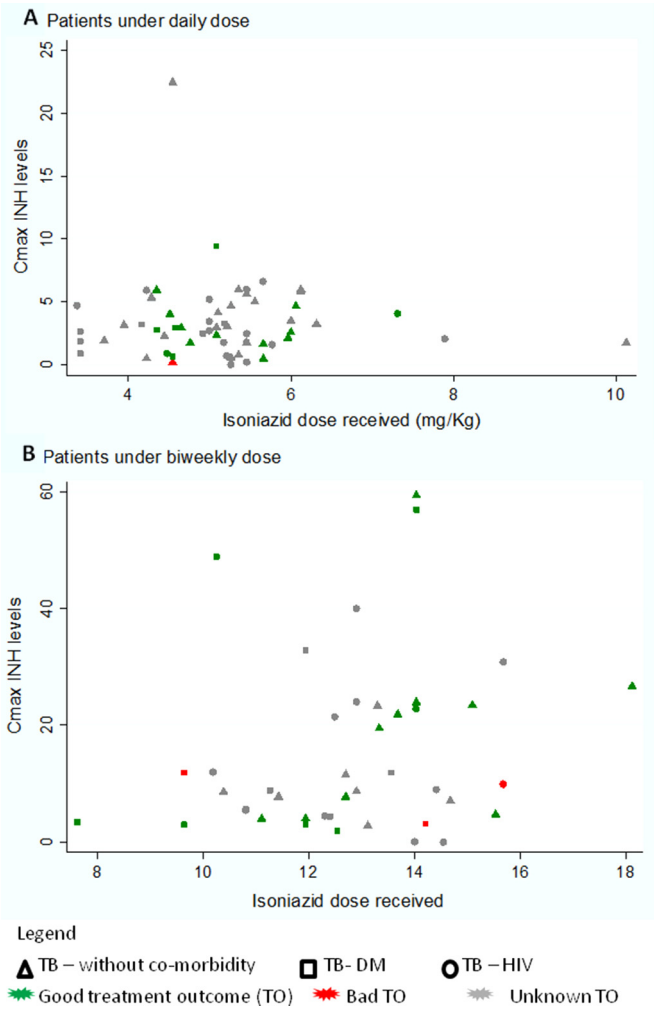

FIG 3 INH C_{max} levels by comorbidity and treatment outcome for patients who received a daily (A) or biweekly (B) dose.

pendent variables were used to further evaluate the effects of the comorbidities of interest, while taking into account the effect of the dose. For the AUC_{0-6} and C_{max}, the isoniazid dose received was the most significant covariate ($P = 0.028$ and $P = 0.029$, respectively), whereas comorbidity (i.e., having HIV infection or DM), age, sex, fasting, and intestinal parasitosis did not significantly influence the PK parameters (data not shown).

The MIC data were unavailable; therefore, the PK-PD parameters were not estimated.

Treatment outcomes were available 6 months after the completion of therapy in 99 of 107 patients. After the exclusion of 10 patients diagnosed with multidrug-resistant TB, 26 who either abandoned treatment or did not properly complete treatment, and 22 others who lacked a microbiologically confirmed diagnosis, 41 patients were included in the treatment outcome subanalysis. A total of 37 patients were regarded as cured, and 4 had an unfavorable outcome; two patients (one who was positive for HIV) died during treatment, one patient had a drug-susceptible relapse within 6 months after an apparent cure, and one patient experienced prolonged treatment for persistent TB despite treatment.

In samples of both intensive and maintenance phase groups, there was no significant difference in the C_{max} of patients with a good treatment outcome compared to patients who had treatment failure or relapse. Three of the four patients who had unfavorable outcomes had very low C_{max} levels when sampled in either the intensive or continuation phase compared to 14/34 (41.2%) of those with favorable outcomes who had very low levels.

DISCUSSION

We aimed to study the INH PK under real-life conditions in the PNTP using sparse PK sampling during daily and twice-weekly dosing. The range of INH doses included in the study was wider than in most published PK studies and clearly demonstrates the linearity of INH PK with weight-adjusted doses.

With the two dose regimens, the INH PK was highly variable, with >30% of patients failing to exceed a C_{max} deemed very low compared with those in other PK studies. A major finding was that most patients during the continuation phase received a weight-adjusted biweekly dose of INH below the PNTP-recommended 15-mg/kg/day dose. This was a particular problem in TB patients with DM who had a higher BMI, but we were unable to determine whether the lack of dose adjustments to account for weight gain during treatment may also have contributed. These observations have since led the PNTP to change the maximum biweekly isoniazid dose in the continuation phase from 800 mg to 900 mg and to reemphasize the importance of weight-based dosing.

We examined several other factors that might also explain the high interindividual variability we observed. Data on fasting prior to the administration of drugs were limited to a subset of patients, but in this underpowered subanalysis, which was not statistically significant, the observed PK parameters were lower for those who did not fast. Neither DM nor HIV independently influenced the INH PK. The DM population in the twice-weekly dosing group showed lower INH exposure, but this was explained by the higher BMIs. The sparse sampling scheme limited our ability to detect changes in the rate of absorption reflected in the T_{max} or C_{max}. If such altered absorption exists, it did not seem to influence the overall AUC_{0-6}, notwithstanding the reduced precision of calculating an AUC using two time points versus more enriched time point sampling. We did not obtain NAT2 genotype or metabolite data to accurately classify individuals by acetylator status. However, the population distribution of isoniazid half-lives was clearly bimodal. Even though the underlying distribution of acetylator phenotype is, in fact, trimodal (24), our data did not support a clear differentiation between intermediate and slow acetylators. More than half of the patients were predicted to be fast acetylators, which may have also contributed to low and variable INH exposure. Little information is currently available on acetylator status in Latin America, especially in Peru, where the *4 allele associated with the fast acetylator phenotype, and which is overrepresented in Asia, may be common (28). Future studies should address this issue with genotyping or metabolite data.

Efficacy targets for PK parameters for anti-TB drugs have historically been based on reference therapeutic ranges derived from achieved PK alone (20). The relevance of these to short- or long-term outcomes remains unclear. There were several studies in the 1960s that related the treatment outcome to PK and particularly to INH concentrations (29–31). Gangadharam et al. showed that the therapeutic efficacy increased above a critical peak concentration of "about 3 μg/ml" of INH (31). Another study supporting these

data showed that early bactericidal activity (EBA) leveled off at a 2-h INH concentration between 2 and 3 μg/ml (32). Weiner et al. also reported low INH concentrations associated with poorer treatment outcome in patients who received once-weekly INH with rifapentine but not in those who received twice-weekly INH with rifapentine (26), which might serve as a surrogate for what should be expected if there was any noncompliance during the daily therapy. On the other hand, most studies of longer-term outcomes have not reported associations with INH PK parameters; instead, pyrazinamide or rifampin is usually implicated (10, 11, 14, 15, 17, 18). In this study, cutoffs suggested in the literature were not associated with poor outcomes. However, the relationship of the INH AUC with drug effect (at least as measured by early bactericidal activity) is well defined (28), which suggests that the variability in PK that we observed might be of clinical significance, especially during intermittent dosing.

Plasma exposure as measured by the median AUC_{0-6} was comparable with the median AUC_{0-12} described for patients with favorable treatment outcomes in two Tuberculosis Trials Consortium (TBTC) studies (28 mg·h/liter in "study 22" [26] and 48.8 mg·h/liter in "study 23" [25]), which used the same biweekly dosing regimen. However, our study lacked the power to detect small changes in important outcomes, such as the emergence of resistance and treatment failure/relapse. Moreover, since we were unable to measure the MICs, which is key for calculating the PK-PD parameters of the bactericidal activity for isoniazid (17), we were unable to evaluate the effect upon clinical outcome.

Conversely, one should also take into account that the combination therapy (with rifampin, ethambutol, or pyrazinamide) might have positively influenced the treatment outcome. Although this synergistic effect can potentially be measured through a microdilution checkerboard assay (33), drug susceptibility testing is done individually for each drug (34). Thus, any effect of a low INH concentration might be overcome by the effect of the other agents and thus not directly influence treatment outcome.

The rationale for the intermittent administration of drugs in the continuation phase is based on *in vitro* studies, which have suggested that antituberculosis drugs, particularly INH, demonstrate a significant and prolonged postantibiotic effect which permits an extended dosing interval (23). While the role of intermittent regimens in the intensive phase of treatment was recently questioned (35), no important differences in treatment outcome were noted in two meta-analyses of clinical trials between daily and intermittent dosing during the continuation phase, whether administration was twice or thrice weekly (6, 36). While updated WHO treatment guidelines no longer promote twice-weekly dosing, this preference appears largely based on consideration of the redundancy of the number of doses in the regimen rather than on the results of PK-PD analysis (5). Our data suggest that weight-based twice-weekly dosing remains a pharmacologically appropriate strategy in this context, and this dosing strategy appears to be quite robust with respect to the important potentially influential patient factors studied here, at least in the PNTP.

We acknowledge that our study had a number of limitations. For logistical reasons, we limited sampling to two time points (2 and 6 h), which facilitated the real-life nature of the work but limited the precision and accuracy of the C_{max}, T_{max}, and AUC_{0-6} estimates. We were also unable to adequately control or reliably measure the concomitant food intake of all the patients. The study lacked power to assess the full impact of PK parameters on long-term outcomes for several reasons. First, in our data set, TB diagnosis was not confirmed in all cases, particularly in HIV-TB patients, and this restricted our clinical outcome analysis to a smaller subgroup than intended (41 patients), which reduced the power of the study to detect such an effect had it been present. Second, no intermediate bacteriological data or susceptibility data for the pathogen, such as the MIC, were available, which should be taken into account to predict clinical efficacy. Larger studies using more sophisticated sparse-sampling strategies would be needed to better understand the relationship between INH PK and long-term outcomes. In this real-life PK study, variability in INH exposure was driven largely by weight-adjusted dose, a relatively high proportion of fast acetylators, and a possible food effect, whereas important comorbidities, such as DM and HIV, had no demonstrable additional impact on INH PK. Although the C_{max} was low in up to 30% of the participants, the AUC_{0-6} values were comparable with those of other studies and were not associated with poor outcomes. In particular, despite high interindividual variability in exposure, twice-weekly weight-adjusted dosing of INH appears quite robust with respect to important patient factors under program conditions.

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We have no conflicts of interest to declare.

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AUTHOR REPLY

Reply to "Adequate Design of Pharmacokinetic-Pharmacodynamic Studies Will Help Optimize Tuberculosis Treatment for the Future"

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We thank Sturkenboom et al. (1) for their interest in our study and their comments. The pragmatic study design based on two sampling points, 2 and 6 h after drug intake, reflected the real-world approach taken in this study, sampling patients attending a government clinic rather than in the controlled setting of a pharmacokinetic study suite. This sampling strategy has frequently been used in the past (2), and there are data to suggest that the peak serum concentration (C_{max}) occurs between 1 and 2 h postdose when isoniazid is given on an empty stomach (3, 4). If only isoniazid is being measured, a two-sample strategy with estimation of 1- and 4-h concentrations may effectively capture both the C_{max} and the majority of delayed absorption (5). However, isoniazid is usually administered with other drugs which are somewhat more slowly absorbed (e.g., rifampin). In such cases, a 2- and 6-h-postdose sampling strategy facilitating study of both agents seems reasonable (5). In our study, the objective was to determine the pharmacokinetics of isoniazid and also rifampin, and thus the C2-C6 sampling strategy was chosen. Although a more intensive pharmacokinetic sampling would better capture the true C_{max} values (6), as suggested by the authors, the logistical demands of this approach would have compromised study recruitment in most of our community clinic field sites, rendering the study unfeasible.

We agree that it is likely that our data underestimates the true C_{max} and AUC, though there is no reason to believe that values would differ between groups. However, one factor that may delay the time at which C_{max} occurs (T_{max}) is to take the drug with food, in particular with high-fat meals (5). Thus, it is recommended that isoniazid be given on an empty stomach. In our study, we were unable to adequately control or reliably measure the concomitant food intake of all the patients, and this fact may have influenced the final results. In this regard, another study is being undertaken with an aim to reliably control food intake with the drugs of patients, and at the same time, the pharmacokinetics of TB drugs will be measured at three time points: 2, 4, and 6 h after the drug intake. Larger studies like this one are required; using sparse population pharmacokinetic sampling schemes in different settings would be desirable, though these probably require a greater level of research infrastructure than is currently available in public clinics in Lima, Peru.

Regarding treatment outcome, in this subanalysis, we included only 41 drug-susceptible patients and excluded patients diagnosed with multidrug-resistant TB, patients who either abandoned treatment or did not properly complete treatment, and others who lacked a microbiologically confirmed diagnosis. However, we agree that our study was not designed to relate the treatment schedule strategy to treatment outcome and that larger studies with long-term follow-up are required to provide relevant data in this respect.

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PLOS ONE

RESEARCH ARTICLE

Robust and Reproducible Quantification of the Extent of Chest Radiographic Abnormalities (And It's Free!)

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Abstract

Rationale

Objective, reproducible quantification of the extent of abnormalities seen on a chest radiograph would improve the user-friendliness of a previously proposed severity scoring system for pulmonary tuberculosis and could be helpful in monitoring response to therapy, including in clinical trials.

Methods

In this study we report the development and evaluation of a simple tool using free image editing software (GIMP) to accurately and reproducibly quantify the area of affected lung on the chest radiograph of tuberculosis patients. As part of a pharmacokinetic study in Lima, Peru, a chest radiograph was performed on patients with pulmonary tuberculosis and this was subsequently photographed using a digital camera. The GIMP software was used by two independent and trained readers to estimate the extent of affected lung (expressed as a percentage of total lung area) in each radiograph and the resulting radiographic SCORE.

Results

56 chest radiographs were included in the reading analysis. The Intraclass correlation coefficient (ICC) between the 2 observers was 0.977 ($p<0.001$) for the area of lung affected and was 0.955 ($p<0.001$) for the final score; and the kappa coefficient of Interobserver agreement for both the area of lung affected and the score were 0.9 ($p<0.001$) and 0.86 ($p<0.001$) respectively.

Conclusions

This high level of between-observer agreement suggests that this freely available software could constitute a simple and useful tool for robust evaluation of individual and serial chest radiographs.

Introduction

Chest radiographs (CXR) provide valuable information regarding extent and progression in many respiratory diseases. Accordingly, for the study of specific illnesses such as occupational lung diseases the utility of chest radiology has been greatly improved by the application of standardized reading methodology[1]. Different methodologies, such as the Chest Radiographic Reading and Reporting System[2], have been proposed to standardize CXR reading for TB and other lung diseases, and also for grading the severity of CXR abnormalities[3].

Chest radiography is a rapid examination suitable for on-site interpretation with a high sensitivity when any abnormality is considered[4]. However, the heterogeneous CXR manifestations of pulmonary TB can lead to inconsistencies in CXR interpretation. Similarly CXR reading is somewhat subjective, so CXR interpretation is highly reader-dependent which can contribute to inter- and intra-observer differences[5,6] and is also dependent upon the expertise of the reader[7]. There have been several attempts to automate reading of CXR by computers[4,8] although it is challenging, particularly due to the low specificity[4].

Recently a simple method for grading chest radiography (CXR) severity in adults diagnosed with sputum smear positive pulmonary tuberculosis (TB) was designed and validated, and shown to correlate with baseline and clinical and microbiological severity and response to treatment[9].

This is likely to be of particular relevance for the evaluation of CXR in clinical trials, where precise, accurate and reproducible data is particularly important. A simple equation was generated to develop the CXR score as follows: proportion of total lung affected (%) + 40 if cavitation is present. This score was able to predict 2-month sputum smear status. To grade the percentage of affected lung, visual estimation of the extent of opacification, cavitation or other pathologies as a percentage of visible lung fields is made.

However, as Ralph *et al* acknowledge, a significant limitation of this method is the low rate of inter-observer agreement in CXR assessment which was low overall, although more substantial agreement was achieved for some variables after adjusting kappa values for variable prevalence and reporter bias[9]. The concordance among the total amount of lung affected was 0.85 (95% limits of agreement 28.2% -22.46%).

Moreover, the poor agreement between radiologists and clinicians has been also reported elsewhere[5,6].

This difficulty (in reproducibly estimating extent of radiographic abnormaliy) can be overcome using novel radiologic software which is capable of accurately measuring a determined area of a radiological digitalized image, giving a precise percentage of lung affected instead of a visual estimation. However this software is not usually available in the field and CXRs are often not performed in a suitable digital X-ray system. Using a standard digital camera, a digital picture of a conventional CXR may be obtained although this file is usually not compatible with digital X-ray software. We have developed a simple methodology based on free image editing software (GIMP, http://www.gimp.org/), which can read any type of digitalized image and provides simple capability to measure selected areas of an image. The objective of this sub-study was to evaluate the reproducibility of lung area estimation using this tool in tuberculosis patients.

Methods

Study methods

In a study of TB drug pharmacokinetics patients diagnosed with and treated for pulmonary TB in south Lima, under the DOTS programme of the Peruvian National TB programme, were invited to participate from July to December of 2009. As part of this study, a CXR was performed to all patients to assess cavitation and extent of the disease. All CXR films were digitalized into

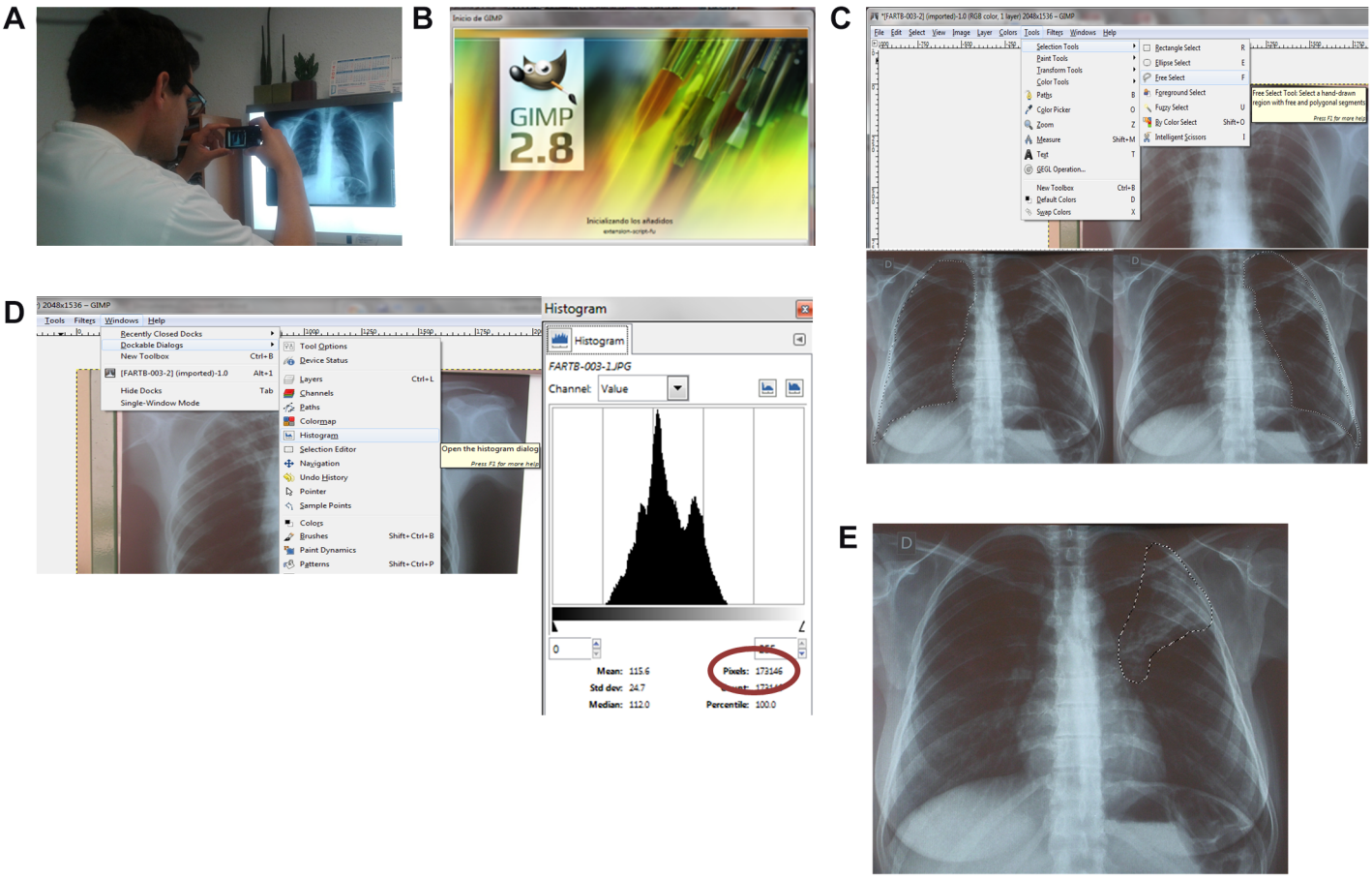

Fig 1. Flowchart of the followed methodology. A. Health worker taking a photo of a radiograph. B. Opening JPEG files with GIMP software. C. Selection of the area. D. Pixel quantification. E. Selected "affected area."

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JPEG files by taking a photograph with conventional digital camera (See Fig 1). The digital image capture was performed by the same person, with the same camera and in the same place for all the CXRs. All CXR films were the same size and the distance from the digital camera to the films was established when the LCD monitor or the viewfinder of the camera framed the whole image. The zoom was not used in order to retain the maximum resolution of the image.

CXRs were coded and stored in a computer at the laboratory offices of Universidad Peruana Cayetano Heredia (UPCH).

Ethics statement

The study protocol and the consent form were approved by the ethics committee of UPCH and Dirección de Salud-II (DISA II) Lima Sur (regional Ministry of Health). All patients gave written informed consent to participate in the study. The individual from the picture (Fig 1A) in this manuscript has given written informed consent (as outlined in PLOS consent form) to publish these case details.

CXR evaluation

Two independent raters, blinded to the other's scores, evaluated the CXRs of study participants. Both were physicians specialized in Internal Medicine with more than 8 years of experience in

clinical practice. For the CXR reading, they opened the JPEG files of each radiograph using the free software GNU Image Manipulation programme (GIMP 2.8). The GIMP software is available, with installation instructions, at: http://www.gimp.org/downloads/

Before commencing data collection, the two researchers involved in the study received brief training of 30 minutes about the use of the GIMP software, specifically about how to use the different commands of the software. This software permits determination of a selected area of an image by measuring the number of pixels enclosed in the selected area. The procedure is as follows:

1. > Free selection tool command (a command that permits to select a determined area), (see Fig 1B) then use the mouse to draw a polygon around area of interest.
2. > Dockable Dialogs > histogram (the command that permits to measure this area in pixels) (see Fig 1C), a determined number of pixels is obtained.
3. Enter data into a simple excel spreadsheet with built in equations that automatically calculate percentage of lung area affected and the score.

Accordingly, the pixels of a selected "affected-lung "area (See Fig 1D) can be compared with the pixels o the total area of lungs (this would be the 100%) in the radiography (see Fig 1B) and the percentage of the lung affected can be calculated using a simple rule of three.

This methodology was applied to evaluate the lung affected area in each radiograph and derive a number representing the percentage of lung affected. Readers judged whether cavitation was visualized and added 40 if this was the case, to determine the final score for each radiograph, according to the method developed by Ralph et al,[9]. A data-base model for data-entry can be found in Tables A and B in S1 File.

Statistical analysis

The agreement between the raters was calculated using an intraclass correlation coefficient (ICC) with a two-way mixed model and with 95% confident interval. ICC was interpreted as poor (0–0.5), moderate (0.5–0.75), good (0.75–0.9) and excellent (0.91–1) according to Portney [10]. Moreover, the variable "Proportion of lung affected" was categorized into 4 different levels of lung affectation: <25%, 25–50%, >50–75% and >75%. The "score" variable was also categorized into 4 different levels: <12.5, 12.5–25, >25–50 and >50. In both cases, the interobserver agreement (IOA) beyond chance was evaluated by calculation of kappa coefficient. Data were analysed using STATA ver. 12.

Results

60 participants were included in the TB – pharmacokinetic study although the CXR was only performed in 56 which were therefore included for the purpose of this study. The raw data can be found in Table C in S1 File. ICC between the 2 observers was 0.923 (0.872–0.954, $p<0.001$) for the determination of the total area of the lungs (pixels) of each CXR and 0.977 (0.961–0.986, $p<0.001$) for the area of the lung affected. ICC was 1 when the presence of cavitation was evaluated. When the final composite score was determined, the ICC between the 2 raters was 0.995 (0.991–0.997, $p<0.001$) (Fig 2). Kappa coefficient for IOA of the score was 0.86 ($p<0.001$) and when the proportion of lung affected was evaluated, kappa coefficient was 0.9 ($p<0.001$).

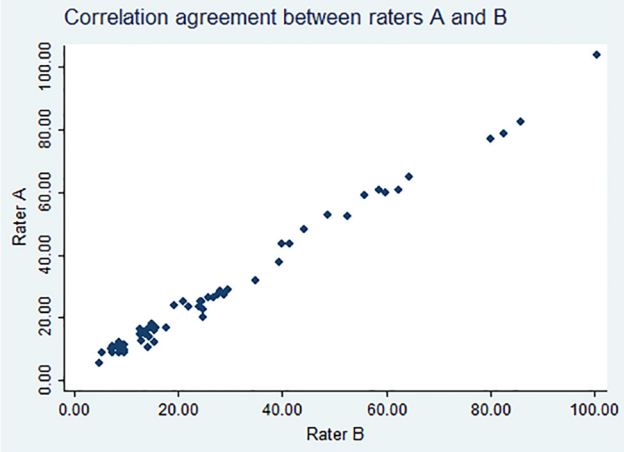

Fig 2. Intraclass correlation agreement between rater A and B.

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Discussion

An objective, reproducible and standardized interpretation of chest radiographs for the detection of active pulmonary tuberculosis is crucial in the final evaluation of the severity of the disease and assessment of therapeutic response and the need for a universal and standard system for CXR reporting in TB is acknowledged[9]. The score developed by Ralph *et al.* is indeed a simple tool that can be used where a numerical score is required for the purpose of comparing radiographic severity between adults with smear-positive pulmonary TB and also to monitor an individual's improvement over time (e.g. to assess drug efficacy in clinical trials). However, calculation of this score requires an assessment of the proportion of lung affected which is subject to significant inter-observer variability [6]. A more robust and reproducible way to define this metric would be very helpful. In health settings where digital systems to perform CXR have been implemented this drawback is substantially reduced since novel software specific for CXR reading permits selection and measurement of polygons or areas. However, such facilities are frequently not available in the majority of resource-constrained countries with the highest burden of TB.

We propose that this alternative tool in which the hard copy chest radiograph film can be captured with a simple digital camera and then read by free software to measure affected areas of the image provides a useful tool for objective, reproducible assessment. Any image processing software could then be used. The high inter-observer agreement of 2 different raters, clinicians but not experienced radiologists, demonstrates the applicability of this tool in the objective interpretation of chest radiographs in a setting representative of clinical practice.

We acknowledge that the lack of an external reference standard with which to compare the observers' ratings may be regarded as an inherent limitation of our study design. However, demonstrating the reliability and agreement does not require such an external 'gold standard' as comparisons are done between and within observers, rather than with an external reference standard, as in diagnostic accuracy studies[11].

Conclusions

Our findings demonstrate excellent inter-observer agreement in the interpretation of the extent of chest radiographic abnormality in smear-positive pulmonary TB patients. The use of the free and simple-to-use GIMP software should be considered when it is desirable or necessary

to quantify the affected proportion of the lung (acknowledging the two-dimensional nature of a CXR). Similarly it may be helpful for both single use or serial review of CXR severity scoring in adults with smear-positive pulmonary TB.

Supporting Information

S1 File. Table A: Instructions for completion of the spreadsheet to calculate proportion of lung affected. Table B: Database model. Table C: Database of the study.
(XLSX)

Acknowledgments

We are indebted to Xose who gave us the idea of using this free-software for the evaluation of radiographs.

Author Contributions

Conceived and designed the experiments: ARM DAM. Performed the experiments: ARM EA. Analyzed the data: ARM DAM. Contributed reagents/materials/analysis tools: ARM EA DAM JM. Wrote the paper: ARM EA DAM JM.

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¿Una absorción reducida de rifampicina e isoniacida contribuye a una respuesta desfavorable al tratamiento de la Tuberculosis?

Cuestionario

01. Nombre _____ 02.Nº identificación _____
02. Sexo __ (Hombre=1, Mujer=2) 03.Fecha Nacimiento __ /__ /____

ANTECEDENTES (0=No, 1=Si, 2=Desconocido, 9=no apuntado)

04. Cirugía digestiva __
05. En caso afirmativo [tipo cirugía] __
1=Gástrica, 2=Resección Intestinal, 3=Resección colon, 4=otros
06. Especifica otros _____
07. Malabsorción __
08. En caso afirmativo (etiología malabsorción_____
09. Gastroenteritis reciente __ (3 o más depos/día durante >3 días en 15 los últimos 15 días)
10. Parasitosis intestinal previa __
11. Otros _____
12. Diarrea crónica (3 o más deposiciones/día durante más de 15 días) __
13. Tratamiento actual_____
14. ¿Está hospitalizado? __
15. En caso afirmativo, causa: _____

TUBERCULOSIS

16. Forma de tuberculosis predominante __
1=Pulmonar, 2=Pleural, 3=Ganglionar, 4=Meningitis, 5=Abdominal, 6=Osteoarticular, 7=Cutánea, 8=Diseminada, 9=Otros

17. Especifica otros _____
18. Fecha inicio del tratamiento __ /__ /____

Tratamiento (0=No, 1=Si, 2=Desconocido, 9=no apuntado)
19. Isoniacida __ 23. Estreptomicina __
20. Rifampicina __ 24. Quinolona __
21. Piracinamida __ 25. Otros __
22. Etambutol __ 26. Especifica otros _____

27. Fase de tratamiento __
1=6 días a la semana, 2=2 veces por semana
28. Observaciones (situación) _____

29. Dosis rifampicina (mg/día) ___
30. Dosis Isoniacida (mg/día) ___

31. Observaciones _____

32. Peso al inicio del tratamiento (Kg) ___ 32. Altura (cm) ___

DIABETES

33. Diabetes __ (0=No, 1=Si, 2=Desconocido, 9=no apuntado)

34. Fecha de diagnóstico __ /__ /_____

Tratamiento para diabetes: (0=No, 1=Si, 2=Desconocido, 9=no apuntado)

35. Dieta __ 38. Sufonilureas __ 41. Otros __
36. Metmorfina __ 39. Repaglinida __ 42. Especifica otros_____
37. Glitazonas __ 40. Insulina __ _____

Compliaciones de la Diabetes: (0=No, 1=Si, 2=DK, 9=missing value)
43. Retinopatia __ 46. Gastroparesia __
44. Neuropatia __ 47. Nefropatía __
45. Vasculopatia __

HIV

48. HIV __ (0=No, 1=Si, 2=Desconocido, 9=no apuntado)
49. Fecha del Diagnóstico __ /__ /_____
50. Cd4 _____
51. Fecha __ /__/ _____

52. Tratamiento antiretroviral # (0=No, 1=Si, 2=Desconocido, 9=no apuntado)

53. Lamivudina __ 58. Nevirapina __ 63. Especifica Otros
54. Abacavir __ 59. Lopinavir __ _____
55. Zidovudina __ 60. Atazanavir __ _____
56. Estavudina __ 61. Ritonavir __ _____
57. Efavirenz __ 62. Otros __ _____

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64. control de Hiperglicemia HbA1C _____

65. Niveles séricos de rifampicina a las 2 horas _____
66. Niveles séricos de rifampicina a las 6 horas _____

67. Niveles séricos de isoniacida a las 2 horas _____
68. Niveles séricos de isoniacida a las 6 horas _____

PARÁSITOS (0=No, 1=Si, 2=Desconocido, 9=no apuntado)

69. Parásitos intestinales __

70. Parásitos no patogénicos __
71. Parásitos patogénicos __

Cuestionario inicio del estudio (1) 01.Nº identificación PEGATIN

¿Una absorción reducida de rifampicina e isoniacida contribuye a una respuesta desfavorable al tratamiento de la Tuberculosis?

2. INICIALES _____
3. Sexo __ (Hombre=1, Mujer=2) 4. Fecha Nacimiento __ /__ /____
5. Centro procedencia _____
6. Fecha visita __ /__ /____

ANTECEDENTES (0=No, 1=Si, 2=Desconocido, 9=no apuntado)

07. Cirugía digestiva __
08. En caso afirmativo [tipo cirugía] __
1=Gástrica, 2=Resección Intestinal, 3=Resección colon, 4=otros
09. Especifica otros _____
10. Malabsorción __
11. En caso afirmativo (etiología malabsorción_____
12. Parasitosis intestinal concomitante __
13. tipo parásito _____
14. Diarrea crónica (3 o más deposiciones/día durante más de 15 días) __
15. Tratamiento actual_____
16. ¿Está hospitalizado? __
17. En caso afirmativo, causa: _____
18. Test VIH __
19. Resultado __ (0=negativo, 1=positivo, 2= indeterminado, 3=desconocido, 9= no apuntado)

TUBERCULOSIS

20. **Situación TB** __
(1=Nuevo, 2=Recaída temprana (<6 meses), 3=Recaída tardía (>6 meses), 4=desconocido, 9=no apuntado)
21. Tiempo de **evolución de la enfermedad** desde la aparición de los síntomas (semanas) __

Síntomas (0=No, 1=Si, 2=Desconocido, 9=no apuntado)

22. **Pérdida de peso** (Más del 10% del peso corporal) __

Cuestionario inicio del estudio (1) 01.Nº identificación PEGATIN

23. **Tos** __ 24. **Fiebre** (>38ºC) __
25. **Dolor torácico** __ 26. Sensación de **ahogo** __
27. **Hemoptisis** (tos con sangre) __ 28. **Sudoración** __
29. Pérdida de **apetito** __ 30. **Otros** __
31. Define otros _____

32. **BK positivo** (1= 1 cruz, 2= 2 cruces, 3= 3 cruces, 4= desconocido, 9= no apuntado) __
33. Fecha **primer BK positivo** __ /__ /_____ (de este episodio clínico, sea del centro que sea)
34. Fecha **inicio del tratamiento** __ /__ /_____

Tratamiento (0=No, 1=Si, 2=Desconocido, 9=no apuntado)
35. **Isoniacida** __ 36. **Rifampicina** __
37. **Piracinamida** __ 38. **Etambutol** __
39. **Estreptomicina** __ 40. **Quinolona** ___
41. **Otros** __ 42. Especifica otros _____

43. Dosis rifampicina (mg/día) ___
44. Dosis isoniacida (mg/día) ___
45. dosis ethambutol (mg/día) ___
46. Dosis pirazinamida (mg/día) ___
47. Observaciones _____
48 **Marca** de la pastilla _____

49. **Peso** al inicio del tratamiento (Kg) _____ 50. **Altura** (cm) ___

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51. **Rx torax ___**
0= Normal, 1= Infitrado lobar, 2= Afectación bilobar, 3= Afectación bilateral, 4= Cavitación, 5=desconocido, 9= no apuntado (Definir SCORE)

52. **MODS inicial**: __ (0= Neg, 1= pos, 2=desconocido, 9=no apuntado)
Sensibilidad MODS inicio (0=resist, 1= sensible, 2= desco, 9=no apunta)
53. R ___ 54. H _____
55. **Cultivo convencional** (INS) __ (0= Neg, 1= pos, 2=desco, 9=no apunt)
Sensibilidad proporciones (0=resiste, 1= sensible, 2= desco, 9=no apunta)
56. **R** ___ , 57. **H**___, 58. **E** ___, 59. **P** ___.

Cuestionario seguimiento (2) 1. Nº identificación PEGATIN

¿Una absorción reducida de rifampicina e isoniacida contribuye a una respuesta desfavorable al tratamiento de la Tuberculosis?

2. **INICIALES** _____ 3. **Fecha** visita __ /__ /____

4. **Visita** nº ___ (1=dosis 26, 2= dosis 50)

5. **Sintomatología** ____

(0=Empeoram, 1= Igual, 2= leve mejoría, 3= franca mejoría, 4= asintomático)

6. Esquema terapéutico ___

(0= sigue esquema 1, 1=sigue esquema 1 pero se alarga fase intensiva, 2= cambio a esquema por MDR, 3=cambio de esquema por RAFA, 4=desconocido, 9=no apuntado)

Medicación: (0=no, 1=Si, 2=desco, 9= no apunt)

7. **R** ___, 8. **H**___, 9. **P**___, 10. **E**____, 11. **Quinol**___, 12 **PAS** ___, 13. **Cicloserina**__ , 14. **Streptomicina**___, 15. Otro **Inyectable**___, 16. **Otros**___

17. Define otros _____

18 Paciente se **excluye** del estudio ___ (0=no, 1=Si, 2=desco, 9= no apunt)

19 **Ayuno** __ (0=no, 1=Si, 2=desconocido, 9= no apuntado)

Efectos secundarios de la medicación

(0=No, 1=Si, 2=Desconocido, 9=no apuntado)

20 Náuseas/vómitos ___

21 Diarrea ___

22 Epigastralgia ___

23 Reflujo ___

24 Otros ___

25 Define otros_____

26 **Peso** _____

---

27 **Auramina** ___

(0= Negativo, 1= 1cruz, 2=2 cruces, 3=3 cruces, 4=no realizado, 9= no apuntado)

28 Cultivo **MODS** ___

(0= Negativo, 1= positivo, 2=desconocido, 9=no apuntado)

Cuestionario seguimiento (2) 1. Nº identificación PEGATIN

Cuestionario fin tratamiento (3) 1. Nº identificación PEGATIN

¿Una absorción reducida de rifampicina e isoniacida contribuye a una respuesta desfavorable al tratamiento de la Tuberculosis?

2. **INICIALES** _____ 3. **Fecha visita** __ /__ /____
4. **Sintomatología** ____

(0=Empeora, 1= Está igual, 2= leve mejoría, 3= franca mejoría, 4= asintomá)

5. **Fin de tratamiento** ___ (0=no, 1=Si, 2=desconocido, 9= no apuntado)
6. **Fecha** fin de tratamiento __ /__ /____
7. **Nº meses** en tto ___
8. En caso de que se haya alargado el tratamiento (>1 mes), Especificar la causa _____

9. Valoración médica ____ (Fracaso=0, curado=1, continúa tto =2, abandono=3, desconocido=4, 9= no apuntado)

Tratamiento global (nº dosis)

Fase intensiva: 10. R ___, 11. H ___, 12. P ___, 13.E ____, 14. Quinol___

Fase mantenimiento: 15. R ___ 16. H___ 17. P___, 18. E ____ 19. Qui___

Efectos secundarios de la medicación (0=No, 1=Si, 2=Desco, 9=no apunta)

20. Náuseas/vómitos ___
21. Diarrea ___
22. Epigastralgia ___
23. Reflujo ___
24. Otros ___
25. Especificar otros _____

26. **Peso** ____

En caso de que se hayan realizado de forma extra…..

27. **BK** ___
(0= Neg, 1= 1cruz, 2=2 cruces, 3=3 cruces, 4=no realizado, 9= no apunt)
28. Cultivo **MODS** ___ (0= Neg, 1= pos, 2=desconocido, 9=no apuntado)
29. **Rx** de tórax ___ (0=empeoramiento, 1=igual, 2= mejoría, 3= completa resolución, 4=desconocido, 9= no apuntado)

Cuestionario final (4) 1. Nº identificación PEGATI

¿Una absorción reducida de rifampicina e isoniacida contribuye a una respuesta desfavorable al tratamiento de la Tuberculosis?

Nota: Completar también en aquellos pacientes excluidos del estudio por el motivo que sea.

2. **INICIALES** _____
3. **Fecha visita** __ /__ /____
4. **Sintomatología** ____

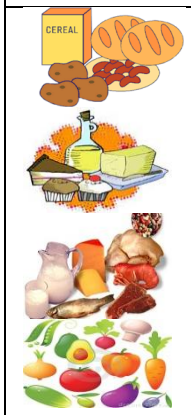
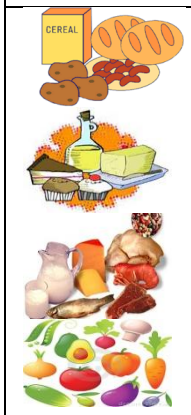
(0=Nunca mejoró, 1= Mejoró pero vuelve a tener síntomas, 2= A Asintomático, 3=paciente no contactado, 9=no apuntado)

5. **Datos TB ___**

Paciente sensible curado=1,
Paciente MDR inicial (excluido del estudio)=2,
TB sensible al inicio y MDR tras iniciar tto=3,
Fracaso sin ser MDR=4,
abandono de tto >1 mes=5,
Paciente perdido=6,
Recaída temprana <6 meses=7
Desconocido =8,
No apuntado=9

5. Comentarios:_____
_____

SEMANA Nº …….. PACIENTE Nº ……..

	Lunes	Martes	Miércoles	Jueves	Viernes	Sábado
 HORA						
 HORA						
 HORA						