Pulmonary tuberculosis case detection in a medium incidence middle-income country

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Abbreviations

AFB	Acid Fast Bacilli
BCG	Bacille Calmette-Guérin
CI	Confidence Interval
DALY	Disability Adjusted Life Years
DOTS	Directly Observed Therapy Short Course
DST	Drug Susceptibility Test
EQA	External Quality Assurance
HIV	Human Immunodeficiency Virus
IRB	Institutional Review Board
IUATLD	International Union Against Tuberculosis and Lung Disease
LQAS	Lot Quality Assurance Sampling
LTBI	Latent Tuberculosis Infection
MDG	Millennium Development Goals
MDR	Multidrug Resistant
MDR-TB	Multidrug Resistant Tuberculosis
MGIT	Mycobacteria Growth Indicator Tube
MODS	Microscopic Observation Drug Susceptibility
NAAT	Nucleic Acid Amplification Test
NTP	National Tuberculosis Program
OR	Odds Ratio
TB	Tuberculosis
TST	Tuberculin Skin Test
WHO	World Health Organization
XDR	Extensively Resistant Tuberculosis

Samenvatting

Opsporing en afdoende behandeling van mensen met longtubercoluse (tbc) is essentieel voor de bestrijding van de ziekte. Gemiste of laattijdige diagnoses leiden tot langere periodes van besmettelijkheid, die de tbc-transmissie in stand houden. Modellering toont aan dat opsporen van 70% van de gevallen van longtuberculose met een positief sputumuitstrijkje en genezen van 85% ervan – de huidige doelstellingen van de Wereldgezondheidsorganisatie (WGO) – tbc-incidentie kan doen dalen met 10% per jaar. In 2014 werden echter wereldwijd slechts 60% van de geschatte gevallen met positief uitstrijkje ontdekt en daalde de tbc-incidentie daalde met slechts 2%.

Het meeste onderzoek naar tbc-opsporing is biomedisch en gericht op ontwikkelen van nieuwe, meer performante diagnostische testen. De implementatie van deze testen heeft evenwel nauwelijks impact gehad op de tbc-incidentie. Deze thesis combineerde een gezondheidssysteem en een operationeel perspectief om het bredere proces van gevalsopsporing te onderzoeken. We hebben in Lima, Peru, zes studies rond opsporing van pulmonaire tbc en multiresistente (MDR) tbc uitgevoerd: vertrekkend van de selectiecriteria voor verdachte, te testen gevallen, over hun identificatie en de testprocedure zelf, tot aan het begin van behandeling. De studies hadden tot doel evidentie te genereren over de prestaties en tekorten in deze stappen en mogelijke oplossingen voor verbetering voor te stellen. De studieomgeving was San Juan de Lurigancho, een district in de periferie van Lima met meer dan een miljoen inwoners, waar de prevalenties van tbc en MDR-tbc tot de hoogste van het land behoren.

Om de efficiëntie van de diagnose van longtuberculose door middel van microscopisch onderzoek van sputumuitstrijkjes te verbeteren, evalueerden we eerst de gevalsdefinitie van “mensen verdacht op tbc” – personen die gedurende 14 dagen of meer hoesten – en het toepassen van deze definitie. In transversaal onderzoek bij 4376 personen die een sputumstaal voor tbc-diagnose hadden ingediend, bepaalden we het percentage positieve uitstrijkjes en de associatie tussen positiviteit en de duur van hoest, de kenmerken van de patiënt en gezondheidszorgkarakteristieken. Meer dan de helft van de patiënten wiens sputum getest was (55,3%, 2418), rapporteerden hoest <14 dagen. Van hen waren er 3,2% (78) positief versus 12,4% (243/1958) in diegenen met hoest ≥ 14 dagen. De onafhankelijke determinanten van een positief uitstrijkje waren hoesten voor ≥ 14 dagen, doorverwijzing door gezondheidswerkers, bezoek aan een tweedelijns

gezondheidszorginstelling, mannelijk geslacht en leeftijd tussen de 15 en 44 jaar. De inclusie van personen die niet beantwoordden aan de gevalsdefinitie van “verdacht op tbc”, was het gevolg van een handelswijze gedreven door een prestatiecriterium opgelegd door het tbc-programma, namelijk dat elke zorginstelling verondersteld wordt om sputummicroscopie uit te voeren bij 5% van de volwassen patiënten die op raadpleging komen. Onze aanbeveling is dat criterium bij te stellen en de huidige definitie van “verdacht op tbc” te blijven gebruiken omwille van haar hoge rendement, maar het is wel essentieel om de naleving ervan te borgen.

In een tweede studie over tbc-diagnose door microscopisch onderzoek wijzigden we de procedure voor selectie van de uitstrijkjes voor externe kwaliteitszorg (EKZ) van het laboratoriumwerk. In tien gezondheidscentra werden gedurende twee jaar de opvolgingsuitstrijkjes van patiënten onder behandeling geselecteerd in plaats van uitstrijkjes voor diagnose. Vals-negatieve fouten kwamen vaker voor bij opvolgingsuitstrijkjes dan in uitstrijkjes voor diagnose: 25 (3.5%) vs. 3 (0.6%). De gevoeligheid om kwaliteitsproblemen op te sporen werd dus aanzienlijk verhoogd en het gewijzigde steekproefschema, dat kleinschaligere steekproeven mogelijk maakte, bleek zeer efficiënt en effectief om laboratoria die ondermaats presteerden te identificeren. We zagen echter geen noemenswaardige verbetering van de prestaties in het tweede jaar van de studie. Dit was een gevolg van suboptimale feedback van EKZ-resultaten naar de lokale laboranten toe door de supervisoren. We bevelen aan om de EKZ met onze aangepaste steekproefstrategie op te zetten en om het toezicht en het feedbackproces binnen het laboratoriumnetwerk te versterken.

Om de gevalsopsporing in specifieke groepen met verhoogd tbc-risico te verbeteren, hebben we eerst een longitudinale studie uitgevoerd in huishoudcontacten van recent gediagnosticeerde tbc-patiënten. Bij de 5466 huishoudcontacten van 1178 patiënten spoorden we tbc-incidentie binnen twee jaar na de blootstelling op en we identificeerden kenmerken van huishouden, patiënt en contact die een verhoogd risico bepalen. De tbc-incidentie onder contacten binnen het huishouden was 1918 (95% BI 1669-2194) per 100.000 manjaar, meer dan tien keer hoger dan in de algemene bevolking. Tbc kwam meer dan zes maanden na diagnose in het indexgeval voor in 121/205 (59,0%) gevallen in huishoudcontacten. Aantal bacillen in het uitstrijkje en de tijd tussen de eerste symptomen en de start van de behandeling in het indexgeval, alsook de verwantschap met het indexgeval en het geslacht van het contact waren significant geassocieerd met tbc-

incidentie in de contacten. Onze aanbeveling is de routineopvolging van huishoudcontacten te verlengen van de huidige zes maanden tot twee jaar na de diagnose van een indexgeval. Zich toespitsen op een deel van de contacten binnen het huishouden op basis van risicofactoren kan de efficiëntie verhogen, maar de haalbaarheid en aanvaardbaarheid hiervan voor patiënten, contacten en personeel moet bepaald worden.

Een tweede studie in de groepen met verhoogd tbc-risico betrof mensen die leven met hiv/aids. We bepaalden de diagnostische nauwkeurigheid van een commerciële geautomatiseerde nucleïnezuuramplificatie-test (Xpert®MTB/RIF) bij hiv-positieve personen verdacht op tbc. Bij 131 hiv-positieve patiënten met een sterk vermoeden op longtuberculose, werd de opsporing van tbc door Xpert®MTB/RIF vergeleken met een referentiestandaard samengesteld uit cultuur in Löwenstein-Jensen (LJ) en in vloeibaar medium. De gevoeligheid van de Xpert MTB/RIF was 97,8% (95% CI 88,4-99,6) (44/45), de specificiteit 97,7% (95% CI 91,9-99,4) (84/86) en de positief voorspellende waarde 95,7% (95% CI 85,5-98,8) (44/46). Xpert®MTB/RIF ontdekte 13/14 tbc-gevallen met negatief uitstrijkje, en overtrof daarmee het microscopisch onderzoek (p = 0,0002). We bestudeerden ook de detectie van rifampicine-resistentie. De gevoeligheid hiervoor van Xpert®MTB/RIF was 100% (95% CI 61,0-100,0) (6/6) en de specificiteit 91,0% (95% CI 76,4-96,9) (30/33). Samengevat, Xpert®MTB/RIF scoorde goed voor tbc-diagnose en deed het beter dan microscopisch onderzoek van uitstrijkjes, maar de specificiteit voor de detectie van rifampicine-resistentie was laag. Wij bevelen uitrollen van Xpert®MTB/RIF aan voor de diagnose van tbc en resistente tbc in hiv-positieve patiënten, ter vervanging van de minder presterende nitraatreductase assay die momenteel gebruikt wordt.

Om de opsporing en het opstarten van de behandeling van multiresistente tuberculose (MDR-tbc) te verbeteren, evalueerden we eerst de gevalsdefinitie van MDR-tbc-verdachte. We voerden een transversale studie uit om het percentage MDR-tbc te bepalen onder tbc-patiënten met geen van de risicofactoren gebruikt door het tbc-programma om geneesmiddelengevoeligheidstesten uit te voeren (gekend contact met een tbc-patiënt wiens behandeling is mislukt of die gestorven is of bij wie MDR-tbc vastgesteld werd; immunosuppressieve co-morbiditeit;
ex-gevangene; gevangenis- en gezondheidszorgpersoneel; en alcohol- of drugsmisbruik). We vonden 6,3% (95% CI 4,4-8,3) (37/584) MDR-tbc onder tbc-patiënten die niet routinematig zouden getest worden voor geneesmiddelengevoeligheid. Dit wijst erop dat patiënten testen voor MDR-tbc op basis van de momenteel gebruikte risicofactoren

ontoereikend is in omgevingen met aanzienlijke MDR-tbc-prevalentie, want er worden veel gevallen gemist. We bevelen aan om een bredere definitie van MDR-tbc-verdachte te gebruiken of, indien mogelijk, universeel te testen op MDR-tbc bij alle tuberculosepatiënten. Algemeen testen zal een vroege start van een adequate behandeling en een correcte aanpak van contacten mogelijk maken.

Vervolgens voerden we een retrospectieve studie uit bij patiënten bij wie MDR-tbc bevestigd was, om het oponthoud tussen de diagnose van MDR-tbc en de aanvang van de behandeling te bepalen. We constateerden dat de tbc-diagnose zich niet onmiddellijk vertaalde in behandeling wanneer MDR-tbc werd vermoed of bevestigd. Bij 35% (13/37) van de patiënten bij wie onmiddellijk MDR-tbc-therapie opgestart werd, verstreken meer dan 30 dagen tussen het eerste resultaat van een positief uitstrijkje en de start van de behandeling. Bij 27% (24/88) van de patiënten die overschakelden op MDR-tbc-therapie, verstreken meer dan 30 dagen tussen de laatste dosis van de standaardbehandeling en de eerste dosis van de MDR-behandeling. De operationele processen en stappen nodig om geïndividualiseerde MDR-tbc behandelingen in te stellen en beschikbaar te maken in de perifere zorginstellingen moeten ingekort worden.

Doorheen onze studies kregen we een meer volledig beeld van het opsporingsproces van longtuberculose in perifere zorginstellingen in een groot district in oost-Lima. Onze resultaten hebben geleid tot aanbevelingen die de opsporing van tbc en MDR-tbc kunnen verbeteren en het opstarten van de MDR-tbc-behandeling kunnen bespoedigen. Systematisch inzicht in het proces van gevalsopsporing kan ook het uitrollen van nieuwe tests, met inbegrip van toekomstige “point-of-care” testen, vergemakkelijken en helpen om hun volledige potentieel te benutten. Vanuit een mondiaal perspectief is onze omgeving – stedelijk, middeninkomen, met een mediaan tbc- en MDR-tbc-probleem en met lage hiv-prevalentie – specifiek. Onze resultaten kunnen niet direct geëxtrapoleerd worden, maar de aanpak die we gevolgd hebben kan elders worden overgenomen om een systematische evaluatie van tbc-gevalsopsporing uit te voeren.

Summary

Case detection is central to tuberculosis (TB) control. Missed or delayed diagnoses lead to longer periods of infectiousness that sustain TB transmission. Modelling indicates that detecting 70% of smear-positive pulmonary TB cases and curing 85% of them - the present World Health Organization targets - can decrease TB incidence by 10% per year. However, in 2014, worldwide, only 63% of the estimated smear-positive cases were detected and notified and TB incidence is declining at only 2% per year.

Most research addressing case detection is biomedical and focuses on developing new diagnostic tests that perform better than the traditional ones. However, implementation of these tests has hardly had an impact on TB incidence. This thesis combined a health system and an operational perspective to investigate the broader process of case detection. We designed and conducted, in Lima Peru, six studies on pulmonary TB and MDR-TB case detection: from the criteria used in the selection of suspects to be tested, to their identification and the testing procedure itself, up to the start of treatment. The studies aimed to generate evidence on performance gaps at these steps and propose potential solutions for improvement. The study setting was San Juan de Lurigancho, a peri-urban district in Lima with over one million inhabitants where TB and MDR-TB prevalence are amongst the highest in the country.

To augment the efficiency of the diagnosis of pulmonary TB with smear microscopy, we first evaluated the case definition of TB suspects– a person coughing for 14 days or more - and the compliance with this case definition. In a cross sectional survey among 4376 persons that had submitted a sputum sample for TB diagnosis, we determined the smear positivity rate and its association with the duration of cough and with patient and health service characteristics. Over half of the patients who had sputum tested (55.3%, 2418) reported cough for <14 days. Of them, 3.2% (78) were smear positive vs. 12.4% (243/1958) of those coughing ≥ 14 days. Cough for ≥ 14 days, being referred by health care staff, attending a secondary-level health care facility, male sex and age between 15 and 44 years were independent determinants of smear positivity. The inclusion of persons who were not TB suspects was a consequence of behaviour driven by a performance target set by the TB program, which requires every health facility to perform smear microscopy in 5% of all adult patients that receive consultations. We recommend

revising that target and continuing to use the current TB suspect definition, as it has a high smear positivity yield, but it is essential to ensure compliance with it.

Subsequently, in a second study on TB diagnosis through smear microscopy, we modified the approach to sampling slides for the external quality assurance (EQA) of laboratory performance. In ten health facilities, during two years, instead of selecting diagnostic slides for EQA, we selected follow-up slides of patients that were on treatment. The false negative errors were more frequent in follow-up slides than in diagnostic slides: 25 (3.5%) vs. 3 (0.6%). Hence, the sensitivity to detect quality control problems was substantially increased when rechecking follow-up slides and the modified sampling scheme, which permitted a smaller sample size, proved very efficient and effective for identifying laboratories with substandard performance. However, we observed no actual improvements in performance in year two. This was a consequence of suboptimal feedback of EQA results to local laboratory technicians by the supervisor. We recommend implementing the EQA with our modified sampling strategy and strengthening the supervision and feedback process within the laboratory network.

To enhance case detection in specific groups at increased risk of TB, we first conducted a longitudinal study in household contacts of recently diagnosed TB patients. We determined TB incidence within two years of exposure among 5466 household contacts of 1178 patients, and identified household, patient and contact characteristics that determine an increased risk. The TB incidence among household contacts was 1918 (95% confidence interval (CI) 1669-2194) per 100,000 person-years, more than ten times higher than in the general population. Incident TB occurred more than six months after the index case's TB diagnosis in 121/205 (59.0%) household contacts. Bacillary load and time between symptoms and treatment initiation in the index case, as well as the kinship to the index case and sex of the contact were significantly associated with TB incidence in the contacts. We recommend extending the routine household contact follow-up from the current six months to two years after the index case's diagnosis. Targeting household contacts on the basis of risk factors may increase efficiency, but the feasibility and acceptability for patients, contacts and TB staff needs to be determined.

A second study on groups at increased risk focused on people living with HIV/AIDS. We determined the diagnostic accuracy of a commercial automated nucleic acid amplification test –Xpert®MTB/RIF- among HIV positive TB suspects. In 131 HIV positive

patients with a high suspicion of pulmonary TB, detection of TB by Xpert®MTB/RIF was compared to a composite reference standard of Löwenstein-Jensen (LJ) and liquid culture. The sensitivity of Xpert MTB/RIF was 97.8% (95% CI 88.4–99.6) (44/45), specificity was 97.7% (95% CI 91.9–99.4) (84/86) and the positive predictive value was 95.7% (95% CI 85.5–98.8) (44/46). Xpert®MTB/RIF detected 13/14 smear-negative TB cases, outperforming smear microscopy ($p = 0.0002$). Detection of rifampicin resistance was also studied. The sensitivity of Xpert®MTB/RIF was 100% (95% CI 61.0–100.0) (6/6) and specificity was 91.0% (95% CI 76.4–96.9) (30/33). In summary, Xpert®MTB/RIF performed well for TB diagnosis and outperformed smear microscopy, but the specificity for detection of rifampicin resistance was low. We recommend the implementation of Xpert®MTB/RIF for TB and drug resistant TB diagnosis among HIV positive patients in replacement of the currently used underperforming nitrate reductase assay.

To strengthen case detection and treatment initiation of MDR-TB, we first assessed the definition of MDR-TB suspect. We conducted a cross-sectional survey to determine the proportion of MDR-TB among TB patients who did not report any of the risk factors used by the TB program to perform a drug susceptibility test (known exposure to a TB patient whose treatment failed or who died or who was known to have MDR-TB; immunosuppressive co-morbidities; ex prison inmates; prison and health care workers; and alcohol or drug abuse). We found 6.3% (95%CI 4.4–8.3) (37/584) of MDR-TB among TB patients that would not be routinely tested for drug susceptibility. This suggests that testing patients for MDR-TB based on the presence of the currently used set of risk factors is insufficient in settings with high MDR-TB prevalence, as it misses many cases. We recommend to use a broader definition of MDR-TB suspects or, if possible, universal testing for MDR-TB in all TB patients. Universal testing will allow early starting of correct treatment regimens and appropriate management of contacts.

Subsequently, to determine the delay between diagnosis and MDR-TB treatment initiation, we conducted a retrospective study in confirmed MDR-TB patients. We found that TB diagnosis did not translate into immediate treatment when MDR-TB was suspected or confirmed. Among 35% (13/37) of the patients directly started on a MDR-TB regimen, more than 30 days elapsed between the first positive smear result at the health facility and the initiation of treatment. In 27% (24/88) of patients switching to a MDR-TB regimen, more than 30 days passed between the last dose of the drug sensitive regimen and the first dose of the MDR regimen. These delays contribute to ongoing transmission of MDR-TB in

the community. Operational processes and steps to design and make individualised regimens available in health facilities should be shortened.

Throughout our studies, we obtained a comprehensive picture of the pulmonary TB case detection process in peripheral health facilities in a large district in eastern Lima. Our results led to recommendations that can enhance overall TB and MDR-TB case detection and speed up MDR-TB treatment initiation. A systematic understanding of the process of case detection can also facilitate the implementation of new tests, including future point-of-care tests and permit to reach their full potential. From a global perspective, our middle-income urban setting with a medium TB and MDR-TB burden and low HIV prevalence is particular. Our results may not directly extrapolate to different settings, but the approaches we used can be replicated elsewhere to conduct a systematic assessment of TB case detection.

1. Introduction

1.1. Tuberculosis: an overview of the disease

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis.* The bacillus identified by Robert Koch in 1882 has a lipidic wall, which is resistant to decoloration with alcohol and acid. This wall makes it more resistant to destruction by macrophages. It is however very sensitive to heat and sunlight. It has a very slow replication rate, which is reflected in the subacute onset of TB disease and the long duration of treatment. The main route of transmission is by air. A person with untreated pulmonary TB, or the much less frequent laryngeal TB, aerosolizes droplets with *Mycobacterium tuberculosis*. These droplets can remain suspended in the environment for hours and be inhaled by another person. *Mycobacterium tuberculosis* then lodges in the distal airways and a cell-mediated immune response develops. A person with a suboptimal immune system will develop progressive primary TB disease. Primary TB is more frequent among young children who have not been previously exposed and who develop parenchymal disease. A competent immune system usually contains the infection and it evolves into an asymptomatic, non-infectious state - latent TB infection (LTBI) - which can remain as such for life (1). However, 10% of immune competent persons with LTBI will reactivate into a secondary TB disease or active TB: 5% will do so in the first two years after infection and 5% in the rest of their lifetime. The main determinant of activation of a LTBI is the immune status of the person. Persons with HIV, malnutrition, diabetes mellitus, chronic renal failure and those of very old age have a higher risk of activation. Among these, HIV is the strongest risk factor for activation: approximately 10% of those infected will develop active TB per year (2). In contrast with most opportunistic infections in persons living with HIV, TB occurs at any CD4+ cell count level (3). The risk of activation of LTBI can be reduced with chemoprophylaxis with isoniazid (4). The highest protection is reached when taken daily for twelve months (5). LTBI is relevant for TB control strategies, as it is estimated that a third of the world population is infected and at risk of future activation.

TB has been described in every organ of the human body. Pulmonary TB is the most frequent form comprising between 80 and 85% of all forms. The most common symptoms, which develop insidiously, are cough, weight loss, night sweats and fever. Extrapulmonary TB is more common in children and in immunocompromised adults, the risk is 1.3 more in HIV co infected patients (6,7). Pulmonary TB is the most relevant form

for public health as it is the most infectious one. Patients can get infected at home, at work or in public places, including means of transport. Upon developing symptoms of active TB and reaching a formal health facility, they are screened for TB mainly by assessing the presence of persistent cough and their sputum or other specimen will be tested to confirm or rule out the disease. Most pulmonary TB cases will be diagnosed at primary care facilities. Patients suspected of having extrapulmonary TB, children and patients with co morbidities may be referred to higher level facilities. Patients may not seek care or may attend non-formal health facilities including pharmacies before possibly reaching services providing TB care. Though guidelines recommend single day diagnosis and immediate start of treatment, patients may still get lost during the case detection process and in the end do not start treatment.

Some forms of TB have a worse prognosis such as meningeal TB and miliary TB, a multiorgan form resulting from a large release of bacilli in the blood stream, both of which are universally fatal if untreated (8,9). In contrast, a study in India from the sixties documented that up to 30% of patients with untreated pulmonary TB achieved spontaneous cure and 20% survived, be it chronically ill (10). Treatment with the standard WHO regimen I of anti TB drugs cures over 90% of immunocompetent persons with active pulmonary TB. Standard regimen I consists of two months of six days per week of a combination of isoniazid, rifampicin, pyrazinamide and ethambutol, followed by four months of isoniazid and rifampicin taken either daily or thrice per week. In Peru, up to 10% of patients default treatment (11) and furthermore, repeated and long interruptions without reaching the 30 days without medication, which defines default, are frequent: 17% in the first phase and 40% in the continuation phase of treatment (12).

Standard regimen I is effective for patients with drug sensitive TB, however, *Mycobacterium tuberculosis* can easily develop resistance to any drug. Drug resistance developed soon after streptomycin, the first antibiotic for TB, was used in 1945. The British Medical Research Council conducted trials and found that adding another bactericidal drug (combining streptomycin and isoniazid or isoniazid and rifampicin) reduced the risk of developing resistance. This became the basis of the drug combinations used to treat TB today. Currently, the two most significant forms of drug resistance are multidrug resistant TB (MDR-TB) defined as resistance to isoniazid and rifampicin, and extensively drug resistant TB (XDR TB) defined as MDR-TB plus resistance to a fluoroquinolone and an injectable agent that is used as a second line drug to treat TB. The absolute number of MDR-TB and XDR TB cases is still low but the complexities of diagnosing resistance and

the suboptimal treatment regimens available make it one of the major challenges in TB control. Traditional MDR-TB diagnostic tests take weeks to produce results while new rapid molecular tests can do it in one day, but their implementation is constrained by available resources.

Until very recently, the single MDR-TB treatment regimen recommended had a 18 to 24 months duration with 54% (meta analysis of 9153 individual patients (13)) to 64% (meta analysis of 36 studies (14)) of patients successfully completing treatment. Adverse events and length of treatment resulted in, on average, 17% of patients defaulting MDR-TB treatment. This regimen, which is still valid, should be composed of at least five effective drugs for an intensive phase of 8 months and total treatment duration of no less than 18 months. The five drugs include pyrazinamide and four second-line TB drugs - one chosen from group A (levofloxacin, moxifloxacin or gatifloxacin), one from group B (amikacin, capreomycin or kanamycin), and at least two from group C (ethionamide or prothionamide, cycloserine or terizidone, linezolid, or clofazimine). If a combination of five effective TB drugs cannot be composed as above, an agent from group D2 (bedaquiline, delamanid) and other agents from D3 (p-aminosalicylic acid, imipenem-cilastatin, meropenem, amoxicillin-clavulanate, thioacetazone) may be added. Bedaquiline and delamanid are recommended since 2013 and 2014 and are the first TB drugs with a novel mechanism of action discovered in the last 40 years (15,16). Patients may receive MDR-TB standardized regimens in which drugs from the above groups are selected on the basis of national drug susceptibility test surveys or individualized MDR-TB regimens in which drugs are selected on the basis of the individual's drug susceptibility pattern.

In May 2016, WHO updated its MDR-TB treatment recommendations to include a short regimen of 9-12 months that cures more patients than the 18-24 month regimen (17). The shorter regimen recommended is composed of moxifloxacin, clofazimine, ethambutol and pyrazinamide given for 9 months and kanamycin, isoniazid and prothionamide given during the first 4 months intensive phase. The evidence for recommending this regimen comes from observational studies that compare the conventional 18-24 months regimen with the shorter regimen (18–21). The pooled proportion of cured patients with the short regimen estimated by WHO from published and unpublished data is 83.7% compared to 61.7% with the conventional regimen. The short regimen is now being tested in randomized trial conditions: the STREAM trial is a non-inferiority trial conducted by the Medical Research Council Clinical Trials Unit, University College of London and the

International Union Against TB and Lung Disease. The first stage of the STREAM trial compared the conventional and the short regimen, it has concluded patient inclusion and is currently following them until 2017. The second stage of the STREAM trial started patient inclusion in March 2016 and randomizes patients to four possible arms: the conventional 18-24 months regimen, the 9 month regimen of stage 1 (that includes an injectable, kanamycin), a fully oral 9 month regimen (that includes bedaquiline instead of kanamycin and replaces moxifloxacin with levofloxacin), or a 6 month regimen (that includes bedaquiline and kanamycin and drops prothionamide and ethambutol) (17,22,23). The short regimen is already recommended despite being based on very low quality evidence (as it derives from observational studies while the trials are ongoing) because of the consistent results in different settings and the large benefits for the patients and the health system of using a regimen with a duration at least half of the otherwise recommended. The short regimen can be used in all MDR-TB patients that do not have confirmed resistance or suspected ineffectiveness to a drug in the regimen (excluding resistance to isoniazid), that have not been exposed to any of the drugs of the regimen for more than one month, that do not have intolerance or increased toxicity to one or more drugs in the regimen, who are not pregnant and who do not have extrapulmonary disease. The traditional MDR-TB regimen of 18-24 month duration should be used in patients for whom the short regimen is contraindicated.

1.2. Global burden of tuberculosis

In 2010, TB caused globally the loss of 49,396 disability adjusted life years (DALYs), making it the 13th leading cause of morbidity and mortality, down from the 8th position in 1990. However, this progress hides large differences between settings, age groups and gender: while it was the 124th leading cause of DALYs in high income North America, it was the second one in South East Asia. Globally, it is the second leading cause of DALYs among 15 to 39 year old men (24).

The burden of TB is usually measured in terms of annual incidence and mortality rates. National TB Programmes report notified cases to the World Health Organisation (WHO) which provides estimations for each measure. In 2012, an estimated 8.6 million (range, 8.3-9.0) new TB cases occurred corresponding to an annual incidence of 122 per 100,000 inhabitants. WHO groups 22 high burden countries that account for 81% of the world´s estimated incidence. Twenty six percent of estimated incident cases occurred in India and 12% in China, while the highest incidences per population are reported from South Africa and Swaziland with over 1000 per 100,000 inhabitants. The lowest incidences per

population, less than 10 cases per 100,000 inhabitants are invariably found in high-income countries such as Japan, Canada, Australia, New Zealand, United States and most of west Europe. Since 2002, incidence rates have fallen in every region of the world, though at different speed. Overall, the rate of decline in TB incidence between 2011 and 2012 was 2%. The most spectacular decline in TB was seen in Europe at a rate of 4 to 5% per year. This occurred between 1851 and 1935, before treatment was available but following a dramatic improvement in socio economic and housing conditions (25). The decline went up to 7 to 10% per year with the use of therapy. In 2014, 6 million new TB cases were notified by National TB Programmes to WHO, representing 63% of the estimated cases. The relative contribution of under-diagnosis and of under-notification to the 37% gap in TB case detection is unknown.

HIV coinfection and MDR-TB currently represent the main challenges for TB control. While the burden of TB and of infectious diseases decreased in general in the period 1990-2010, that of coinfection of TB and HIV/AIDS increased (24). The best estimates for the proportion of TB cases that occurred among persons living with HIV was 13% (incidence of 1.1 million out of the 8.6 million) and 75% of the global HIV-TB cases occurred in Africa, mainly in Sub Saharan countries. Of the 1.5 million deaths caused by TB in 2014, 27% occurred among people living with HIV (26).

The burden of MDR-TB in the world is not well characterized and is challenging to measure. (27) Mathematical modelling estimated that in 2012, 3.6% of new cases and 20.2% of retreated cases were MDR. MDR-TB has been described in every region of the world, and while India, China, Russia and South Africa have 60% of MDR-TB cases, the highest proportions of it are in Eastern European countries. For TB and MDR-TB, both absolute numbers and rates are important. For example, the proportion of new TB cases that are MDR in China is 5.7% and in Azerbaijan it is 22.3%, but these account for 18,030 persons and 290 persons, respectively (28).

1.3. Tuberculosis control

TB control aims to reduce human suffering caused by TB by reducing morbidity and mortality. This can be effectively achieved through public health measures that halt the transmission cycle by reducing the number of infectious sources –pulmonary TB cases. To consistently reduce human suffering, TB control programmes also address disease presentations that contribute to a negligible proportion of transmission or none at all such as childhood TB and extrapulmonary TB.

To halt the transmission cycle, TB Programmes organize pulmonary TB case detection by defining criteria for and identifying persons that may have TB (TB suspects) and subsequently testing them for TB. TB cases detected are started on standard treatment regimens that in most cases will render patients non-infectious after a few days, or sometimes weeks, and subsequently cure them. Also, TB programs aim to avoid the generation of drug resistance, a consequence of poor management of drug sensitive TB. Case detection and treatment are the basis of TB control. Modelling indicates that detecting 70% of estimated smear positive TB cases and curing 85% of them can decrease TB incidence by 10% per year (29,30). These are the two main indicators for TB control: the case detection ratio (number of cases notified to the National TB Programme/ estimated cases) and the treatment cure/completion rate (number of patients that were cured plus completed treatment/number of cases detected). Once that target is reached and case detection stabilizes, increasing case detection above 70% would be needed to achieve further reductions in incidence (29). However, the effectiveness of case detection to interrupt transmission depends not only on the proportion of existing cases that are diagnosed and put on treatment but also on the delay between the start of symptoms and treatment initiation. Complementary control activities are chemoprophylaxis for LTBI of children under 5 years old, HIV positive patients and patients with other immunosupressive states, neonatal vaccination with Bacille Calmette-Guérin (BCG) and infection control in health care settings.

Reducing progression of LTBI to active disease with the use of chemoprophylaxis with isoniazid is a secondary activity in terms of public health impact. Despite the fact that a third of the world is estimated to have LTBI and that the pooled protection of isoniazid is 60%, it is not recommended for immune competent adults in high burden countries. The absolute risk of activating the infection is low and the protection is only significant when it is given within 2 years of the infection, an event difficult to establish in populations with frequent exposure except for household contacts. Prophylaxis is however recommended for persons at high risk of progression to active disease such as HIV positive patients or at high risk of developing severe TB such as young children.

All newborns in high incidence countries should be routinely vaccinated with BCG. BCG protects up to 80% against developing TB meningitis and miliary TB, specially in the first five years of life (31,32). Despite being a safe and low cost vaccine, BCG has traditionally been considered to have a minor impact on public health because the level of protection against adult pulmonary TB, the main source of TB transmission, is low and

inconsistent (showing protection in the United Kingdom (rate ratio (RR) of 0.22, 95% confidence interval (CI) 0.16 to 0.31), to no protection in a large trial in India (RR 1.05, 95%CI 0.88-1.25)) (32,33). However in a trial in Brazil, a first dose of BCG to previously unvaccinated school aged children reduced the incidence of TB, especially pulmonary TB, in one of the two cities of the trial (34). The reason for the variation is unclear.

Box. The Stop TB Strategy, 2006 (35)

The Stop TB Strategy Components	
1	Pursue high quality DOTS expansion and enhancement.
	Secure political commitment, with adequate and sustained financing.
	Ensure early case detection, and diagnosis through quality-assured bacteriology.
	Provide standardized treatment with supervision, and patient support.
	Ensure effective drug supply and management.
	Monitor and evaluate performance and impact.
2	Address TB/HIV, MDR-TB and the needs of poor and vulnerable populations.
	Scale up collaborative TB/HIV activities.
	Scale up prevention and management of MDR-TB.
	Address the needs of TB contacts, and of poor and vulnerable populations.
3	Contribute to health system strengthening based on primary health care.
	Help improve health policies, human resource development, financing, supplies, service delivery and information.
	Strengthen infection control in health services, other congregate settings and households.
	Upgrade laboratory networks, and implement the Practical Approach to Lung Health.
	Adapt successful approaches from other fields and sectors, and foster action on the social determinants of health.
4	Engage all providers.
	Involve all public, voluntary, corporate and private providers through public-private mix approaches.
	Promote use of the International Standards for Tuberculosis Care.
5	Empower people with TB, and communities through partnership.
	Pursue advocacy, communication and social mobilization.
	Foster community participation in TB care, prevention and health promotion.
	Promote use of the Patients' Charter for Tuberculosis Care.
6	Enable and promote research.
	Conduct programme-based operational research.
	Advocate for and participate in research to develop new diagnostics, drugs and vaccines.

In 1991 WHO declared TB a global public health emergency and in response launched the “Directly Observed Short Course (DOTS) strategy” and global targets for TB control were set. “DOTS” comprised five components: political commitment, case detection through quality assured smear microscopy, short course treatment, monitoring and regular supply of drugs. The WHO-based Stop TB partnership was founded in 1998. The Stop TB partnership centralizes countries data in an annual report from National TB Programmes,

produces guidelines, generates advocacy and supports National TB Programmes in implementing control strategies. DOTS was expanded to “DOTS-plus” to cover the HIV/AIDS-TB and the MDR-TB epidemics. In 2006, it was further expanded to the “Stop TB strategy” with six components, as detailed in the box, the first of which is the five-component DOTS. The Stop TB strategy endorses the two Millennium Development Goals (MDG) related to TB. In 2015, the Stop TB partnership launched the End TB strategy. The table shows the evolution in the targets set by international organisations for TB control and their status in 2015.

After the introduction of DOTS, the global case detection rate went from 11% in 1995 to 28% in 2000 and then reached 45% in 2003 (36). In 2014 the best estimate was 63% (37). To reach the target of eliminating TB by 2050 (<1 case per million inhabitants), the decline in the global TB incidence would have to increase from the current 2% to 20% per year.

Table. Tuberculosis targets by organization, year launched and status in 2015 (35,36,38)

Organization	Year	Target	Status in 2015
End TB / WHO	2015	By 2035: reduce TB deaths by 95%, cut new cases by 90%	-
Stop TB / WHO	2006	By 2005: case detection >70%, treatment cure >85% By 2015: global burden (prevalence and mortality) are halved relative to 1990 levels By 2050: eliminate TB, global incidence is < 1 case / million population / year	Case detection 63% Cure 87% Mortality reduced by 45% Prevalence reduced by 37% -
Millennium Development Goals	2000	By 2015: halt and reverse TB incidence and TB mortality	Incidence decreases 2% per year Mortality decreased by 45%
WHO/ Global TB Programme	1991	Case detection >70%, treatment cure >85%	Case detection 63% Cure 87%

1.4. The process of tuberculosis case detection

The operationalization of pulmonary TB case detection involves a) defining who has a high pre test probability of TB disease (TB suspects), b) organizing screening by identifying and locating the suspects and have them access health services, c) testing

them for TB once identified, d) linking the diagnosis to treatment initiation and e) monitoring and evaluating the process and adjusting accordingly. The implementation details for each step vary with the local epidemiology and available resources.

The TB suspect definition should allow a diagnosis that is made as close as possible to the day when the patient begins to expel bacilli to contribute to a reduction in incidence, morbidity and mortality (39). A broad sensitive definition of TB (and MDR-TB) suspects might diagnose more cases early, but with low efficiency, while a narrow definition may delay diagnosis of cases sustaining transmission in the community. Persistent cough is a common, easily identifiable symptom of pulmonary TB. The proportion of TB patients found among persistent coughers depends on the background prevalence of TB. WHO defines a TB suspect as a person coughing for three weeks or more. This cut off is considered a good balance between the number of infectious cases detected and laboratory workload (40). Testing patients who are coughing for a shorter duration may include a large proportion of patients that do not have TB. In addition to persons with persistent cough, certain groups are at increased risk of TB as compared to the general population. Contacts of recent TB cases, especially household and close contacts, are an obvious risk group that may be considered TB suspects even if they do not have persistent cough. HIV positive patients may have TB with minimal symptoms; therefore, duration of cough to define a TB suspect is not applicable in HIV populations. In this group, the presence of at least one of the following: cough of any duration, fever, night sweats or weight loss has a sensitivity of 78.9% in detecting a TB patient and a specificity of 49.6% (41).

TB suspects need to be detected and tested for TB. Passive case detection is the strategy most widely used to detect a TB suspect. TB suspects are identified when they present themselves to health facilities looking for symptom alleviation. Such a patient initiated pathway is feasible on the condition that the community is aware that persistent cough is associated to TB and that health care should be sought, and if health facilities are available, accessible and acceptable to the community. TB programmes might or might not include community information and outreach activities in order to sensitize the population with regards to TB. Active case detection, on the contrary, implies a provider-initiated pathway to identify TB suspects, usually outside health facilities (42,43).

MDR-TB cases are detected within the TB case detection process. A MDR-TB suspect is a TB suspect or a TB case with risk factors for MDR. The precise definition of an MDR-TB suspect varies with the local epidemiology. However, some risk factors are global: patients with a persistently positive sputum smear after the fourth month of a drug sensitive TB treatment regimen and contacts of MDR-TB patients are at high risk of MDR-TB. Other common factors that define a MDR-TB suspect are exposure to settings where MDR-TB is prevalent even if there is not a clear exposure to an individual case. These settings are prisons, shelters, health facilities that receive MDR-TB patients, among others. A TB programme can either screen for MDR-TB among groups at risk or universally, in all TB cases. Screening for MDR-TB can be done with rapid drug susceptibility tests (DST) such as Xpert®MTB RIF, Genotype MTB RIF, MODS, among others, and confirmed with traditional DST such as Löwenstein-Jensen or Mycobacteria growth indicator tube (MGIT).

Once detected (identified), the TB suspect should submit two sputa samples. Ideally one of the two should be an early morning sputum, as sensitivity of smear microscopy to detect acid-fast bacilli (AFB) is higher than for on-the-spot sputum. However, in 2011 WHO changed this two-step process of an early morning sputum followed by on-the-spot sputum, to same-day diagnosis with two on-the-spot sputum submitted and analysed the same day (44–46). The same-day diagnosis reduced the number of suspects lost when asked to come back the next day and it reduced sensitivity by only 2.8%. A single positive smear microscopy in any of the two sputa is considered diagnostic of TB in high incidence settings and has a positive predictive value of over 99% (47).

Observation of AFB under the microscope –smear microscopy- is the oldest and still the most widespread diagnostic method for TB. Its sensitivity using a microscope with a light source reaches 60% if correctly implemented, but may be lower in routine conditions. The Ziehl-Neelsen technique, an adaptation of the first method developed in 1900, uses the acid-fast property of the mycobacteria. Carbolfuchsin enters the wall of the bacteria when heated but does not leave when exposed to alcohol /HCL. The stained bacilli are seen in a background of methylene blue. Performing sputum smear microscopy is fast, easy to execute, requires few and affordable lab material, does not necessarily require electricity and permits to rate infectiousness of a specimen. In the Ziehl-Neelsen technique, 1-9 acid-fast bacilli seen per 100 fields is a “scanty positive”, 10-99 AFB per 100 fields is scored “1+”, 1-10 AFB per field is “2+” and >10 AFB per field is “3+”. The

main disadvantage of smear microscopy is its low sensitivity, around 60%. Despite being easy to perform, this non-automated method is particularly prone to the technician's reliability from smear preparation to reading. Finally, microscopy is not specific to *Mycobacterium tuberculosis* as the observed AFBs can also be non-tuberculosis mycobacteria.

Löwenstein-Jensen culture, the TB diagnostic standard of reference for many years, is a low cost test with a moderate sensitivity of 80%, but mainly limited by the very long time needed to obtain results. Growth in solid media can be seen in two weeks but the test cannot be declared negative until 45 days have passed. Löwenstein-Jensen proportion method is the best-known phenotypic method to detect drug resistance. Sputum is either directly inoculated in the medium with antibiotic or indirectly, by inoculating the colonies grown in a culture. A resistant strain is one where microorganisms grow in the presence of a predefined concentration of a drug. Liquid-based mycobacteria growth indicator tube (MGIT) and its commercial versions (BACTEC MGIT 960 system) replaced Löwenstein-Jensen as a faster standard of reference, with the core advantages of reduced time to results and slightly increased sensitivity. Other non-commercial highly sensitive phenotypic tests are the solid-media based thin layer agar (TLA) and the liquid-media based microscopic observation of drug susceptiblity (MODS) test, developed in Peru, (48,49). Both tests detect microscopic growth of *Mycobacterium tuberculosis,* thus reducing time for results compared to Löwenstein-Jensen that detects macroscopic growth. A pooled analysis of turnaround time in 12 studies, found that MDR-TB was detected by TLA in 11.1 days (10·1–12·0) and by MODS in 9.9 days (95% CI 4·1–15·8) (50).

Molecular tests have further reduced the time to diagnose TB and MDR-TB to a few days. Nucleic acid amplification tests (NAAT) amplify *Mycobacterium tuberculosis* specific nucleic acid sequences using a nucleic acid probe that goes to sequences in the rpoB genes for rifampicin and in the katG and inhA genes for isoniazid. However, as only 95% of rifampicin resistant strains and 75 to 90% of low level isoniazid resistant strains have the mutations detected by the tests, these have a lower sensitivity for drug resistance than phenotypic tests. Furthermore, some strains detected by molecular rifampicin-resistant tests are detected as as rifampicin-sensitive by phenotypic tests. Therefore, it is recommend that rifampicin resistance detected by rapid molecular tests is followed by a confirmation phenotypic test. Cepheid® created the GeneXpert platform, an automated

platform that performs a NAAT. Xpert®MTBRIF, the test used in the GeneXpert platform, diagnoses TB and rifampin resistance in 90 minutes. WHO endorsed Xpert MTB/RIF in 2011 as the initial diagnostic test in individuals suspected of having MDR-TB or HIV-associated TB. Its policy update based on three systematic reviews including over 85 papers published since 2011, extends the recommendation to children suspected of having MDR-TB of HIV-associated TB and gives a conditional recommendation, acknowledging resource implications, to use Xpert MTB/RIF as the initial test in adults and children suspected of having TB rather than using conventional microscopy and culture (51). Previous to Xpert MTB/RIF, in 2008, WHO endorsed two commercial line probe assays for rapid screening of patients at risk of MDR-TB: INNO INNO-LiPA Rif.TB® (Fujirebio Europe, Ghent, Belgium) that detects rifampicin resistance and GenoType® *Mycobacterium tuberculosis* drug resistance plus assay (MTBDR*plus*) (Hain Lifescience, Nehren, Germany) that detects rifampicin and isoniazid resistance (52).

Molecular tests, such as Xpert®MTB/RIF and Genotype®MDRTB*plus* are groundbreaking technology because of the rapidity in detecting TB and MDR-TB and the high sensitivity: over 95% for rifampicin and over 90% for isoniazid (53,54). However, the cost of 10 US$ per Xpert MTB/RIF test, for example, is a serious limitation and operational requirements such as stable power, air conditioning, periodic calibration and the need to perform several tests simultaneously may limit its use.

Reliable laboratory results are key to patient management. Quality control is a necessary process to ensure optimal performance of a laboratory. As Ziehl-Neelsen staining is a manual technique, field variations and low reproducibility can reduce sensitivity (55). Traditionally, external quality assurance (EQA) of smear microscopy consisted on rechecking all positive slides and 10% of negative slides. In 2002, an international working group issued guidelines with new EQA procedures that recommended three complementary methods: 1) On-site assessment of laboratory performance applying a standardized questionnaire. The direct contact allows verification of equipment and observation of actual work but it is labor intensive and not necessarily objective. 2) Panel testing with centrally prepared slides that are submitted to local laboratories to be read and to determine concordance of results. This evaluates capability but routine performance and staining and smearing are not evaluated 3) Blinded rechecking of a random sample of slides (positive and negative) from a laboratory. This allows for an objective evaluation of laboratory routine performance (56). These

internationally recommended EQA procedures are included in the guidelines of many national programs, but it is unclear how many programmes are compliant with them. Quality control of culture methods is much less developed and systematic.

The methods described above are the recommended standard to diagnose pulmonary TB and MDR-TB. In case of strong clinical suspicion and a negative culture or NAAT, TB program staff might also request medical evaluation and/or a chest X ray to patients to decide on treatment start. The case detection process concludes once the patient is registered in the treatment register book.

TB screening among HIV positive patients is recommended at all HIV medical visits and at each antiretroviral therapy pick up, by assessing the presence of any one of four clinical symptoms: current cough of any duration, weight loss, fever and night sweats (57). If present, the patient should be tested for TB. The sensitivity of the presence of any of the four symptoms to detect TB in an HIV positive person is 78.9% (41).

The progress in case detection at country and global level is measured through the “case detection rate” which is actually a ratio. It consists of the number of detected TB cases notified by a program divided by the estimated number of incident TB cases. The estimate accounts for the TB cases not diagnosed (or diagnosed by health providers that do not notify them to the surveillance system) and are frequently based on empiric observations and expert opinion. The WHO Global Task Force on TB Impact Measurement has improved the quality of the estimates by developing policy recommendations for the measurement of TB burden. In recent years, at least 16 countries in Asia and Africa have conducted national prevalence surveys. Despite the complexities of measuring incidence and prevalence, high burden countries need to accurately quantify the real burden of TB to be able to design policies and to measure their effect. If a country overestimates its burden, the notified cases will always be below the target and the National TB Programme may take incorrect unnecessary actions to increase case detection. Conversely, if a country underestimates its burden, case detection will be overestimated and the National TB Programme will incorrectly interpret its policies as appropriate. Discrepancies between reality and estimates can be large. In 2007, a prevalence survey was conducted in Eritrea, the first in 45 years in Africa. It found 50 TB cases per 100,000 TB inhabitants, contrasting with the WHO estimates of 251 per 100,000 inhabitants (58).

1.5. TB and its control in Lima, Peru

In 2015, the population of Peru was 31.4 million. Seventy eight percent lived in urban areas and 9.7 millions persons lived in Lima, the capital (59). The gross domestic product per capita was US$ 6,662 of which 4.8% was expended on health. The World Bank classified Peru as an upper middle-income country. From 2004 to 2012, the proportion of the population living in poverty decreased from 52% to 26% and those living in extreme poverty from 21% to 6%. Despite these substantial reductions, large inequalities exist, with poverty concentrated in rural areas and higher income in the coastal urban areas. Peru ranked 77th in the Human Development Index, a United Nations Development Program summary measure to assess countries' achievements in human development beyond economic growth (60).

Life expectancy at birth was 72 years for males and 77.3 years for females, the maternal mortality rate was 66 per 100,000 and the infant mortality rate was 20 per 1,000 live births (59). Peru has a fragmented health system: 20% of the population receive care in the Social Security, the compulsory insurance system for formal employees, 4% has private health insurance and the Ministry of Health provides full subsidized health care to 18% of the population and partially subsidized care to 58% of the population (61).

The WHO region of the Americas, which includes North, Central and South America, is the region with the lowest TB burden and most countries have incidences below 50 per 100,000 inhabitants. In 2012, 99 cases for all TB forms per 100,000 inhabitants made Peru the country with the second highest incidence in the region. For comparison, the incidence per 100,000 in Peru's border countries for the same year was: 127 in Bolivia, 59 in Ecuador, 46 in Brazil, 33 in Colombia, 25 in Argentina and 16 in Chile. Peru has a concentrated HIV epidemic. The prevalence in the 15-49 age group is 0.4% and the proportion of TB patients living with HIV is 2.9% (62). In 2014, the TB case detection rate was 81%. The proportion of treatment success of new smear-positive TB cases was 79% and the proportion of defaulters was 6% (26). MDR-TB proportion among new cases determined in national surveys increased from 3% in the 1999 to 5.3% in 2009 and among retreated cases from 12.3% to 23% (63,64). From 2001 to 2013, 480 cases of extensively drug resistant TB (XDR TB) have been diagnosed in Peru and half of these patients have died. Peru has the largest absolute numbers of confirmed MDR-TB cases and estimated MDR-TB cases among notified cases in the America´s region (37). The causes of the high MDR-TB rates have not been systematically studied and there is no

consensus on them among experts. Among the hypotheses given are the use of the previously WHO recommended regimen II (which added a single drug -streptomycin- to the four drug regimen I) in the late 90s, for patients failing regimen I despite high MDR rates among them, the use until 2013 of rifampin twice weekly in the continuation phase and high rates of default and interruption to treatment.

TB in Peru is an urban disease with 58% of all cases notified in Lima. The surge of population in Lima started in the 70-80's as a result of large migrations from the Andean regions fleeing poverty and the armed conflict in the central and southern Andes. Migrants improvised living places in large areas in the outskirts of town. Many of these slums have now been moderately urbanized, but the areas are still overcrowded and some do not have access to basic services such as water supply. Districts in the north and the east of Lima have the highest TB rates in the country; the other regions with an annual incidence of TB above the national average are Madre de Dios, Ucayali and Loreto in the jungle, and Ica and Tacna on the coast (65).

In 1990, strong political commitment provided budget and administrative support to the first structured approach to control TB. The National TB Programme was set up as a vertical disease control programme with partial integration into general health services (66). DOTS has since been applied in over 99.8% of health facilities in the country. Estimated incidence went from 317 per 100,000 in 1990 to 184 in 2000 to 99 in 2012. The National TB Programme was credited in 1995 by the WHO as a leader in reaching the target of 70% case detection and 85% cure rates (66). Despite this progress, over 20 years after the implementation of DOTS in Peru, the incidence is the second highest in South America. The proportion of cases notified out of those estimated in Peru (the case detection proportion) is reported to be 100% from 2010 to 2012 and 81% in 2013 (26). The denominator is based on the assumption that notification is of good quality and on expert opinion on underdiagnosis, but not on a recent prevalence survey. A prevalence survey is recommended to accurately determine the denominator of the case detection proportion.

In 2005, a health reform dismantled vertical programs and they were fully integrated into general health services. The central activities formerly conducted by the TB program were shifted to what has since been named the "National Strategy to Prevent and Control TB", with limited decision-making and a reduced budget for central managers. Power was shifted to district TB programs and health services and the number of technical

staff reduced. The main advantage of a vertical program is efficiency in obtaining disease specific results. On the other hand, the main disadvantage is that by working in relative isolation, a vertical program does not necessarily contribute to health system strengthening and it may even contribute to fragmentation. There is no evidence on whether the Peruvian health system was strengthened after integration of programs activities to general health services. The National Strategy for TB Prevention and Control is now responsible for TB control in Peru and is supported by the National Institute of Health that oversees the Laboratory Network and the national research priorities. In November 2013, new versions of the TB guidelines were issued after 7 years (67).

Peruvian TB guidelines follow the international recommendations set by WHO and the International Standards of Care (38,68) with minor variations. TB suspects are defined as persons who cough for more than 14 days as opposed to the 21 days recommended by WHO. Passive case detection is implemented in all public health facilities. In the 90's, active case detection was done with mobile teams periodically screening suspects in high burden areas but this was discontinued when incidence decreased. TB suspects have two sputa tested by microscopy using the Ziehl-Neelsen stain. If both are negative, two more sputum smears are requested, one is cultured and the patient undergoes a clinical and radiological evaluation. Until November 2013, DST was done in persons that met one of the criteria to suspect MDR (MDR-TB contacts, contacts of TB cases that failed treatment or died, immunosuppressed TB patients, previously treated TB cases including recurrent episodes, defaulters and failures, health care workers and health sciences students, inmates and prison workers and persons hospitalized for more than 15 days in the two previous years). The National TB Programme has strengthened the laboratory system and scaled up rapid DST and MDR-TB treatment. Seven laboratories perform DST: Löwenstein-Jensen proportion method is performed in all laboratories while Genotype®MDRTBplus (the single rapid molecular test currently recommended in Peru), Griess, and MODS are deployed in different regions of the country (69–72). The new 2013 guidelines recommend universal rapid DST. New patients without drug resistance start a regimen of two months of daily isoniazid, rifampin, pyrazinamide and ethambutol followed by four months of thrice weekly isoniazid and rifampicin. DOTS is health facility-based and a nurse or nurse aid supervises the intake of the medication at the health center and registers it in the patient's treatment card. Attendance is encouraged with free monthly food packages. Treatment for drug resistant TB is individualised. Decentralized expert committees of approximately 10 members design all drug resistant TB treatment regimens

in weekly or bimonthly meetings, depending on the burden of cases in the district. The regimen is designed on the basis of the patient's DST. Liver tests are required at baseline to evaluate potential for adverse events of treatment and a chest physician evaluates all patients before starting treatment. Completing all those requirements can take weeks as they have to be done in different services and facilities and, until 2012, patients had to pay for them. MDR-TB treatment is also provided under facility based DOTS.

1.6. Role of operational research on tuberculosis case detection

Operational research has been defined as the "search for knowledge on interventions, strategies, or tools that can enhance the quality, effectiveness, or coverage of programmes in which the research is being done" (73,74). The gap between the available evidence and routine practice is global. In the United States, 99,000 avoidable deaths occur annually as a result of incomplete or inadequate implementation of evidence-based strategies (75). TB control also suffers from evidence-policy-practice gaps. Successful control of TB in high-income countries was achieved with imperfect diagnostic tools. Strong health systems that make optimal use of available tools and reduction of social inequities may play a more fundamental role than sophisticated diagnostic technology and treatment schemes. Operational research can enhance TB control while affordable improved vaccines, diagnostic technologies and new drugs are being developed and it can facilitate the implementation of innovations as they become available. In 2011, the Stop TB partnership and the Global Fund to Fight AIDS, TB and Malaria formulated operational research priorities (76). The proportion of grants from the Global Fund that included operational research increased from 19% in rounds one to five to 58% in round seven. Studying case detection from an operational perspective could provide evidence that permits increasing the proportion of cases detected above the 63% by using traditional tools as well as using technology in affordable ways for a middle income, moderate TB incidence setting.

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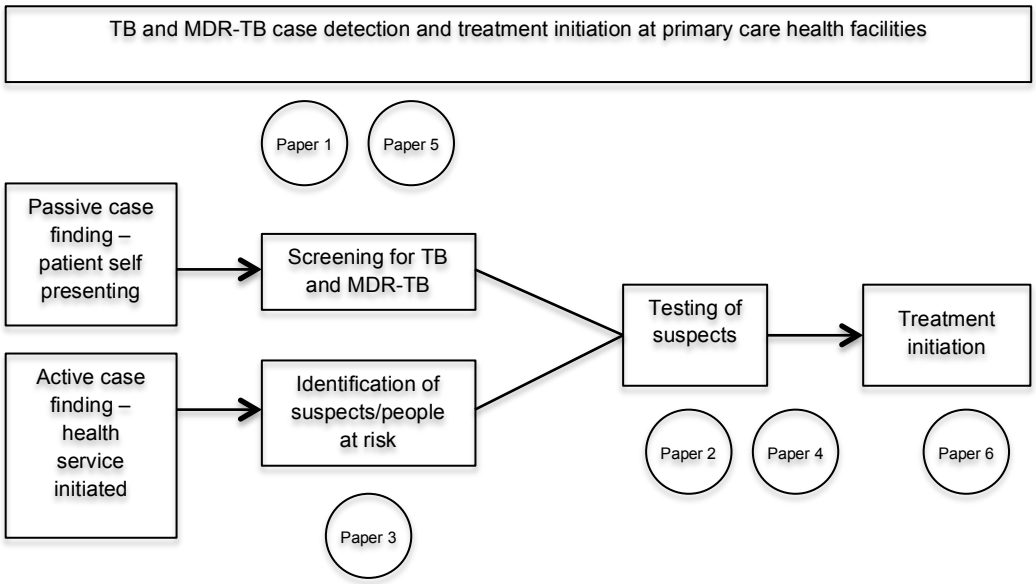
2. Rationale, aim and general methodology of the thesis

2.1. Rationale

This thesis focuses on optimizing the case detection process of pulmonary TB and MDR-TB in Peru, a middle-income country with moderate TB incidence and low HIV prevalence. Despite the increasing availability of moderately well performing diagnostic tests, only 63% of estimated TB cases and 25% of estimated MDR-TB cases worldwide were detected in 2014 (28). Missed, delayed or inaccurate diagnoses lead to treatment initiation delay, to a longer period of infectiousness during which TB transmission continues (63,64) and could lead to poorer clinical outcomes at an individual level. The slowly replicating nature of *Mycobacterium tuberculosis* and the fact that its most common symptoms are non-specific to TB makes case detection a complex process in need of precision and efficacy at each stage, from suspect definition and identification to confirmation of the disease and linkage to treatment.

There is extensive research on evaluation of diagnostic tests, but less so in the process of case detection as a whole. Diagnostic tests will only perform well if implemented embedded in robust case detection systems. An in-depth analysis of the process in place in particular settings and an evidence based evaluation regarding both the barriers to streamlined case detection and those factors that enhance it are urgently needed. This thesis combined a health system and an operational perspective to investigate the broader process of case detection. We designed and conducted, in Lima Peru, six studies on pulmonary TB and MDR-TB case detection: from the criteria from the selection of suspects to be tested, over their identification and the testing procedure itself, up to the start of treatment. The studies aimed to generate evidence on performance gaps at these steps and propose potential solutions for improvement. Strengthening case detection should result in timely and effective treatment of more infectious TB cases and in reduced transmission of TB. The components of the case detection process and where the evidence generated by the thesis fits within the process is presented in the following figure.

Figure. Components of the tuberculosis and multidrug resistant tuberculosis case detection and treatment initiation addressed by this thesis.



Thesis chapters and papers:

3.1 Augment efficiency of diagnosis of TB suspects with smear microscopy
- Paper 1: Duration of cough and yield of smear microscopy
- Paper 2 Quality assurance of smear microscopy by stratified lot sampling

3.2 Enhance case detection in specific groups at increased risk of TB
- Paper 3: Tuberculosis among household contacts of smear-positive tuberculosis cases
- Paper 4: A diagnostic accuracy study of Xpert®MTB/RIF in HIV-positive patients

3.3 Strengthening MDR-TB detection and treatment initiation
- Paper 5: Multidrug resistant tuberculosis in persons with no known risk factors
- Paper 6: Time to initiation of multidrug resistant tuberculosis treatment

2.2. Aim and objectives

This thesis aims to analyse the pulmonary TB case detection process and provide evidence on gaps, inefficiencies and potential interventions that could improve detection to contribute to pulmonary TB control in Lima, Peru.

The specific objectives were:

- To determine the efficiency of diagnosis of TB suspects with smear microscopy

- To determine the potential of enhancing case detection in specific groups at increased risk of TB
- To determine the potential to improve MDR-TB case detection and treatment initiation

2.3. General methodology

We designed and conducted a series of interlinked studies in San Juan de Lurigancho, Lima. Most of the studies were designed and implemented within the framework of a collaborative agreement for research capacity building between the Institute of Tropical Medicine in Antwerp and the Instituto de Medicina Tropical Alexander von Humboldt at the Universidad Peruana Cayetano Heredia in Lima. I was involved in different stages of the studies: in three of them I led the full project from the study design to the writing of the article (paper 3 in chapter 3.2 and paper 5 and 6 in chapter 3.3), in two of them I implemented the studies, analysed the data and wrote the articles (papers 1 and 2 in chapter 3.1) and in one study I collaborated in the design, analysis and writing (paper 4 in chapter 3.2).

San Juan de Lurigancho is the most populous district in Peru, with over one million inhabitants. It is a peri-urban district where 27% of the population lives in poverty. There are variations in the socio economic status within the district: in the upper half, located on the side of a hill, more people live in poverty and in improvised dwellings, while the lower half is urbanised with modern fixed structured housing and fewer people living in poverty.

2.4. Design of the studies included in this thesis

We provide here only a broad overview of the study designs. Full details of all the material and methods can be found in chapter 3.

In chapter 3.1 of the thesis, we focus on smear microscopy, the most widely used diagnostic method for pulmonary TB. **To determine the efficiency in the selection of TB suspects and to test a modified quality control system for smear microscopy,** we first conducted a cross sectional survey on TB suspects identified at all health facilities in San Juan de Lurigancho. We registered patients´ characteristics and routine sputum microscopy results. We defined the optimal cut off point for duration of cough as screening criterion for pulmonary TB and we constructed a logistic regression model to determine the factors associated with smear-positivity. While WHO recommends three-weeks or more of cough for defining a TB suspect, the Peruvian National TB Programme recommends a

two-week cut off and considered reducing it further to one week to increase the number of cases detected. We aimed to determine, for each duration, the smear positivity rate and number of persons to be tested to find a TB case.

In a second study, we modified the sampling technique used for external quality assurance (EQA) for smear microscopy, to reach a feasible sample size of slides to be rechecked for decentralised health facilities. Fixed fraction sampling of slides for EQA rechecking can result in a large sample size. The lot quality assurance sampling (LQAS) technique reduces the number of slides to be rechecked but it can still be considerable where slide positive rates are lower. By focusing on follow up slides instead of diagnostic slides, the probability of errors increases and the total sample size to recheck decreases. We evaluated the performance of the modified EQA in ten selected primary care health centres of San Juan de Lurigancho. The sensitivity to detect quality problems by the proposed method was compared to that of the routine quality control method.

In chapter 3.2 of the thesis we present the results of our research aimed at **determining the potential for enhancing case detection in specific groups at increased risk of TB.** We studied two populations at higher risk of TB than the general population: household contacts of pulmonary TB patients and HIV positive patients. Household contacts share common indoors spaces in the period before an index case is diagnosed in the household and transmission may have occurred. In a prospective longitudinal study, we followed up household contacts to identify those that developed TB within two years of diagnosis of the index case and to determine index case, household, and household contacts' characteristics associated to an increased risk.

HIV positive patients are at higher risk of developing active TB after infection and case detection among them poses additional challenges because they have fewer bacilli in their sputum and their chest X ray can be normal, even in advanced stages of disease. Increasing diagnostic accuracy among them can reduce morbidity and mortality. We conducted a cross sectional study in HIV positive patients attending two referral hospitals in Lima, in order to determine the diagnostic accuracy of an automated real time polymerase chain reaction assay, Xpert®MTB/RIF, in identifying pulmonary TB among TB suspects. The assay was compared to a composite reference standard of solid and liquid culture.

In chapter 3.3 of the thesis, we focus on key aspects of the MDR-TB management: **selection of persons to be screened for MDR and the time between diagnosis and treatment initiation**. Enhancing case detection among MDR-TB cases is key to improving treatment outcomes and to cutting transmission. In Peru, at the time of the study, TB patients were screened for MDR if they reported at least one of a predefined set of risk factors putting them at higher risk of MDR-TB. We conducted a cross-sectional survey among previously untreated pulmonary TB cases with no known risk factors. We determined the prevalence of MDR-TB in this group. Demographic and epidemiological data was obtained to identify factors associated with MDR-TB not included in the risk set routinely used.

MDR-TB diagnosis is only effective in improving treatment outcomes and cutting transmission if the patient is started promptly on effective treatment. The Peruvian National TB Programme and reference laboratory have made substantial efforts to improve MDR-TB management, such as implementation of DST that can provide rapid results and cutting down pre treatment requirements to start MDR-TB treatment. Yet, we hypothesized that times to treatment initiation were still long. We conducted a retrospective study using diagnostic and treatment records of patients with confirmed MDR-TB and extracted the date of the first smear positive result at a health facility and the treatment initiation date for TB and for MDR-TB. We calculated MDR-TB treatment initiation delays.

2.5. Ethical considerations

The protocols for all studies were approved by the institutional review board (IRB) at Universidad Peruana Cayetano Heredia, by the District Health Direction Office of Research at San Juan de Lurigancho. Paper 1 in chapter 3.2 and paper 6 in chapter 3.3 were also approved by the IRB of the Institute of Tropical Medicine in Antwerp, Belgium. All study results were presented to the National TB Programme in meetings and/or technical briefs. DST results from patients in paper 4 in chapter 3.2 and paper 6 in chapter 3.3 were communicated to the treating physician in charge at the health facility where they had been attending. The Ministry of Health provides treatment for TB and MDR-TB free of cost for patients. Data was stored in study databases protected by passwords and in paper files protected by locks in the Tuberculosis Unit at the Instituto de Medicina Tropical Alexander von Humboldt at Universidad Peruana Cayetano Heredia. Names and identification data was stored separately with additional encryption. Data were coded prior to analysis to protect anonymity of participants.

3. Results

3.1. Augment efficiency of diagnosis of TB suspects with smear microscopy

Otero L, Ugaz R, Dieltiens G, González E, Verdonck K, Seas C, Van Deun A, Gotuzzo E, Van der Stuyft P. Duration of cough, TB suspects' characteristics and service factors determine the yield of smear microscopy. *Tropical Medicine and International Health* 2010; 15(12):1475-1480

Otero L, Van Deun A, Agapito J, Ugaz R, Prellwitz G, Gotuzzo E, Van der Stuyft P. Quality assurance of smear microscopy by stratified lot sampling of treatment follow-up slides. *International Journal of Tuberculosis and Lung Disease* 2011; 15(2): 211-216

Paper 1

Duration of cough, TB suspect's characteristics and service factors determine the yield of smear microscopy

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Summary

Objective

To determine the efficiency of routine tuberculosis (TB) case detection by examining sputum smear positivity for acid-fast bacilli in relation to duration of cough, characteristics of TB suspects examined and health service factors.

Method

We combined patient interviews with routine data from laboratory registers in 6 health care facilities in San Juan de Lurigancho district, Lima, Peru. A TB case was defined as a TB suspect with at least one positive sputum smear. We calculated adjusted odds ratios with 95% confidence intervals for the association between smear positivity and health service and patient's characteristics.

Results

Smear positivity was 7.3% (321/4376). Of the 4376 adults submitting sputa, 55.3% (2418) reported cough for <14 days. In this group, smear microscopy yielded 3.2% (78/2418) positive results vs. 12.4% (243/1958) in patients coughing for 14 or more days. Having cough for >2 weeks, being referred by health care staff, attending a secondary-level health care facility, male sex and age between 15 and 44 years were independent determinants of smear positivity.

Conclusions

Routine case detection yields a low proportion of smear-positive cases because of the inclusion of a high proportion of patients without cough or coughing for <2 weeks. Adherence to the national TB control programme guidelines on the selection of TB suspects would have a positive impact on the smear positivity rate, reduce laboratory costs and workload and possibly improve the reading quality of smear microscopy.

Introduction

World Health Organisation (WHO) aims to detect at least 70% of infectious tuberculosis (TB) cases and cure 85% of detected cases as this is considered a cost-effective policy to control TB (Currie et al. 2005). In high-incidence countries, the lack of resources limits the implementation of new and improved diagnostic techniques; even culture can often not be routinely performed (WHO 2009a). In these settings, smear microscopy constitutes a simple, inexpensive and fast method for TB diagnosis which requires minimal equipment – a microscope – that can also be used for other diagnostic tests.

WHO guidelines recommend passive case detection. Two sputum specimens are collected from pulmonary TB suspects, who are persons with persistent cough for at least 2 weeks (WHO 2009b). Specimens are submitted to microscopic examination for acid-fast bacilli (AFB) through Ziehl–Neelsen staining. This screening is meant to increase the probability of ascertaining a TB case and is considered an efficient trade-off between maximal detection of infectious cases and acceptable workload for TB programs (WHO 1998). Screening of the general population is not encouraged, even in high-incidence countries. Diagnostic evaluation for pulmonary TB in a person without a persistent cough is a clinical, not a public health, activity.

For optimal results of sputum smear AFB examination, good-quality sputum samples and adherence to standard laboratory procedures are required, in addition to adequate selection of patients (Luelmo 2004). This assumes the existence of an efficient quality assurance system and properly trained staff. Work overload and inappropriate or incorrectly applied procedures result in a low quality of AFB smear microscopy and fewer TB cases detected (Van Cleef et al. 2003).

The strengthening of Peruvian National TB Program (NTP) since 1990 has had a positive impact on TB control. TB incidence has steadily fallen since 1992 and, by 2006, it had dropped to 110 per 100 000 inhabitants (Suárez et al. 2001; Peruvian National Tuberculosis Program 2006). The directly observed therapy short course (DOTS) strategy now covers more than 99% of public health services in Peru. The NTP guidelines define TB suspects, called respiratory symptomatics, as patients coughing for 14 or more days.

In 2005, 2.1 million diagnostic sputum smears were read in Peru; by 2006, this

had increased to 2.5 million (Peruvian National Tuberculosis Program 2006). However, the proportion of positive diagnostic sputum smears in the same period decreased from 2.3% to 1.7% (Peruvian National Tuberculosis Program 2004). The large number of slides read could have affected the quality of AFB smear examination because of work overload in general laboratories and may reflect non-compliance with guidelines in the selection of TB suspects. To test this last hypothesis, we studied the characteristics and the duration of cough of patients tested for TB in Lima and related these to the smear-positive proportion.

Material and methods

Study setting

The study was conducted in 6 health care facilities in San Juan de Lurigancho, a semi-urban district in Northern Lima. In 2005, national HIV prevalence in TB patients was 1.8%, and San Juan de Lurigancho had a TB incidence of 213 per 100 000 inhabitants (Peruvian National Tuberculosis Program 2005). Five of the facilities are first-level health care centres. These centres offer outpatient services for general medicine, maternal and childcare, laboratory tests for general blood biochemistry and urine and AFB smear microscopy. At the sixth facility, the only second level centre for the upper San Juan de Lurigancho area, inpatient care, mainly for newborns and infants, is also provided, and appendectomies, caesarean sections and other uncomplicated surgical procedures are performed there. This facility also has a pulmonary physician on its staff, carries out X-rays, performs TB cultures and is the reference facility for all the first-level centres. Smear microscopy is carried out using the Ziehl–Neelsen staining method. Routine quality control of AFB microscopy was provided by the district laboratory network.

The NTP has designated offices in all centres. A nurse and a nurse aid are permanently posted there, supervised by a physician who also has other duties in the centre. The NTP staff is responsible for the detection, notification and follow-up of TB suspects, who can consult spontaneously or can be referred by other personnel from the centre. The nurses evaluate the patients, request AFB smear microscopy when indicated and instruct the patient on sputum production. They also supervise DOT (including multidrug-resistant TB treatment) and keep the patient records.

When a TB suspect was detected, two sputa (spotmorning) were requested. If at least one sputum sample was positive (‡1 AFB), the patient immediately started TB

treatment. Only if both smears were negative and symptoms persisted, then the TB suspect was referred for medical examination, two additional samples were collected, one of which was cultured, and a chest X ray was requested. While awaiting the culture results, a clinical-radiological TB diagnosis could be made by the physician, generally a pulmonary physician.

The NTP set for all health facilities a TB screening target of 5% of the adults consulting for any reason (Peruvian National Tuberculosis Program Guidelines 2006). This is the proportion of TB suspects expected given the estimated national TB incidence rate. The target is 6% for high-risk areas such as San Juan de Lurigancho. The proportion of TB suspects detected and screened for TB is evaluated each quarter and constitutes a key indicator of health centre performance for TB control in Peru. Health facilities in the San Juan de Lurigancho district generally reached the quarterly target and screened on a yearly basis, indeed 6% of the adults that consulted (Peruvian National Tuberculosis Program 2005).

Data collection

From August 2003 to September 2005, data were collected at the selected health care facilities. The researchers did not interfere with routine patient management to obtain an operational perspective of the TB case-detection process. Two nurse aids were trained as field workers and posted in the NTP premises at the 6 health care facilities. Each one was assigned to 3 health care facilities, spent 5 days a week in one of them and then rotated to the next. Every patient of 15 years or older who submitted a sample for AFB microscopy was invited to participate in the study. The field workers registered routinely reported data (sex, age, date of sputum submission, diagnostic or follow-up visit and sputum sample number) and additional information (duration of cough, spontaneous presentation or referral by health personnel) as reported by the patient. Subsequently, they obtained the smear results directly from the laboratory AFB register.

Statistical analysis

Data were entered in Epi-Info 3.4 (CDC, Atlanta, GA, USA) and analysed with spss for Windows version 13.0 (SPSS, Inc., Chicago, IL, USA). The unit of analysis was the TB suspect, and the outcome variable was smear positivity. Smear positivity was the proportion of TB cases (defined as at least one positive smear result) of all TB suspects

who submitted sputum samples during the study period. Proportions in subgroups were compared using Pearson's chi square test, and odds ratios (OR) were calculated with 95% confidence intervals (95% CI). To obtain adjusted OR, we constructed a logistic regression model by iterative backward elimination and forward inclusion. All variables that were statistically significant ($P < 0.05$) in the univariate analysis, as well as non-significant variables of interest and plausible interaction terms, were considered for inclusion in the model.

Ethical considerations

This study was approved by the Institutional Ethics Committee at Universidad Peruana Cayetano Heredia. All data were processed anonymously.

Results

During the study period, we enrolled 5,946 patients who submitted 1, 2 or 3 samples in the 6 health care facilities. A total of 4,376 (73.6%) patients that submitted sputum samples for diagnostic purposes were included in the analysis. Of these, 87.5% (3831/4376) submitted two sputum samples and 6.3% (276/4376) submitted three samples. The remaining 1570 submitted a follow-up sample and were already receiving TB treatment. Overall, there were 7.3% (321/4376) smear positive patients. Mean age of the population was 33.3 years (SD ± 15.7) for all the patients. No age difference was observed by smear results: 29.9 years (SD ± 12.5) for the smear-positive patients vs. 33.6 years (SD ± 15.9) for the smear-negative patients. Male patients accounted for 47.4% (2075/4376) of the population but predominated among the smear-positive patients, 61.1% (196/321).

Duration of cough had an asymmetric distribution with a median of 10 days (IQR 0–15) for all patients; 15 days (IQR 15–29) for smear-positive patients, and 10 days (IQR 0–15) for smear-negative patients.

The proportion of screened patients stratified by duration of cough and smear positivity, and the number of patients to be tested to obtain a positive case is shown in Table 1. Of the patients tested, 55.3% (2418/4376) did not conform with the Peruvian NTP definition of a TB suspect, and 83.9% (3673) had less than 21 days of cough. Smear positivity increased from 1.5% in patients reporting no cough at all to 15.5% for persons coughing for 21 days or more, and from 3.2% in non-TB suspects to 12.4% in TB suspects as defined by the NTP. Examining eight persons coughing for 14 or more days would yield

one TB case, while 31 persons would have to be tested to find one TB case if these had cough from one to 13 days.

Table 1. Relation between smear positivity and duration of cough in adults. San Juan de Lurigancho, Lima, 2003-2005

Duration of cough (days)	Patients screened	N° of positive cases	Smear positivity % (95%CI)	Odds ratio (95%CI)	N° of persons tested to find one positive case % (95%CI)
	N=4376 (100%)	321	7.3 (6.6)		13.6 (12.2–15.1)
0	1156 (26.4)	17	1.5 (0.8–2.2)	1	67.9 (35.9–100.0)
1-6	348 (8.0)	15	4.3 (2.2–6.4)	3.0 (1.5–6.1)	23.3 (11.7–34.7)
7-13	914 (20.9)	46	5.0 (3.6–6.5)	3.5 (2.0–6.2)	20.0 (14.3–24.5)
14-20	1255 (28.7)	134	10.7 (9.0–12.4)	8.0 (4.8–13.3)	9.4 (7.9–10.9)
≥21	703 (16.1)	109	15.5 (12.8–18.2)	12.3 (7.3–20.7)	6.4 (5.3–7.6)
<14	2418 (55.3)	78	3.2 (2.5–3.9)	1	31.0 (24.2–37.8)
≥14	1958 (44.7)	243	12.4 (11.0–13.9)	4.1 (3.2–5.4)	8.1 (7.1–9.0)

Table 2 shows the variables associated with smear positivity. Ninety-seven of the 4,367 patients were excluded from this analysis because of missing data on referral status. Duration of cough was the factor most strongly associated with smear positivity (AOR 6.3, 95% CI 4.2–9.4). Attending a second-level health care centre also had a strong association (AOR 3.1, 95% CI 1.9–4.9). Referral for smear microscopy by health staff, sex and age were further independent predictors. We found an interaction between the effect of duration of cough on smear positivity and the level of health facility consulted: the effect of duration of cough was stronger in the five first-level health centres than in the second-level centre. We did not find an interaction between duration of cough and referral by health staff, or between level of service consulted and referral.

Discussion

In this study, more than half of the patients screened for TB in a semi-urban district in northern Lima did not comply with the NTP operational definition for a TB suspect (i.e. patients coughing for 2 or more weeks). Moreover, the rate of smear positivity in this group (3.2%) was quite inferior to the one in the group fulfilling such definition (12.4%). Our definition of a TB case was a patient with at least one positive smear, without considering culture results, which is the definition currently in use by the NTP to start TB therapy. Thus,

it allows evaluation of the routine case detection process, and it gives our study direct programmatic implications.

Table 2. Determinants of smear positivity in adults screened for TB. San Juan de Lurigancho, Lima, 2003-2005.

Determi-nant	Smear positive	Smear negative	Crude OR (95%CI)	P	Adjusted OR (95%CI)	P
Sex						
Female	124 (5.5)	2132 (94.5)	1	<0.0001		<0.0001
Male	196 (9.7)	1827 (90.3)	1.8 (1.5-2.3)		1.8 (1.5–2.4)	
Age (years)						
15-29	197 (8.7)	2067 (91.3)	1	0.0002		<0.0001
30-44	80 (7.7)	960 (92.3)	0.87 (0.67–1.14)		0.84 (0.63–1.11)	
45-59	31 (5.0)	583 (95.0)	0.56 (0.38–0.82)		0.54 (0.36–0.81)	
>60	12 (3.3)	349 (96.7)	0.36 (0.19–6.53)		0.26 (0.14–0.47)	
Referral by health staff						
No	153 (6.0)	2415 (94.0)	1	<0.0001		<0.0001
Yes	167 (9.8)	1544 (90.2)	1.7 (1.4–2.1)		1.8 (1.4–2.3)	
Duration of cough (days)						
0-13	78 (3.3)	2259 (96.7)	1	<0.0001		<0.0001
≥14	242 (12.5)	1700 (87.5)	4.1 (3.2–5.4)		6.3 (4.2–9.4)	
Level of service						
First	167 (6.2)	2512 (93.8)	1	<0.0001		<0.0001
Second	153 (9.6)	1447 (90.4)	1.6 (1.3–2.0)		3.1 (1.9–4.9)	
Interaction term 2nd level of service & duration of cough ≥14 days					0.47 (0.27–0.80)	0.0058

The proportion of smear positivity in a population depends on the prevalence of TB, accessibility of the health services, selection criteria for the patients to be screened and the quality of the test execution (Harries et al. 1997; Makunde et al. 2007).

In our study population, the rate of smear positivity was 7.3%, which points to suboptimal efficiency: In high incidence settings relying on passive detection of TB cases, 10% smear positivity is considered indicative of optimal performance (Baily et al. 1967; Rieder et al.1997). The low smear positivity rate found in our study is a consequence of inadequately selecting individuals with short duration of cough for screening. This has also been observed in other studies (Baily et al. 1967; Harries et al. 1997; Makunde et al.

2007).

We did not find a significant difference in the smear positivity proportion of the patients coughing for 14 or more days [12.4% (95% CI 11.0–13.9%)] vs. those coughing for 21 or more days [15.5% (95% CI 12.8–18.2%)]. An earlier diagnosis is made in the former scenario which could have a positive impact on transmission. These findings support the use of 2 weeks of cough as a cut-off point for the definition of a TB suspect in high incidence settings, as recommended by the Peruvian NTP. In a multicentric study in India, Santha et al. (2005) also compared the cut-off points of 2 vs. 3 weeks of cough and found 12% and 13% smear positivity, respectively. In another study in India, 8.8% and 9.7% of positive smears were found in persons coughing for 2 and 3 weeks, respectively, while only 0.4% was found in those who had only 1 week of cough (Baily et al. 1967). A large study in eight rural regions of Tanzania found proportions of smear-positive cases varying from 14.3% to 23.8% in persons coughing for 3 or more weeks (Ipuge et al. 1996). Smaller proportions of smear-positive cases have been described in other settings where the prevalence of TB is lower and/or other definitions of TB cases are used (Banda et al. 1998; Shargie et al. 2006; Bastos et al. 2007; González-Ochoa et al. 2009).

Although it would have been interesting, we could not, unfortunately, investigate the reasons for the large number of non-TB suspects who submitted a sputum sample. Patients with other symptoms compatible with TB or epidemiological criteria to suspect TB might justifiably have been tested without persistent cough. However, those reasons can hardly explain why more than 50% of those screened did not comply with the definition of TB suspects. This large proportion may have been a consequence of health care workers' will to reach the target of screening 6% of all adult outpatients in high risk areas, set by the NTP in 2000 as an indicator of good performance and still applicable today. Imposing such target with almost fixed resources and under declining TB prevalence rates could be counterproductive and place an excessive workload on health staff. Patients with <14 days of cough who are screened are wrongly registered as TB suspects attending a health centre, which biases the TB indicators.

Adherence to the still current NTP definition of TB suspect (cough ‡14 days) would reduce laboratory workload and increase the positive predictive value of smear microscopy, even if a small number of patients that do have TB would not be diagnosed before 2 weeks of cough. We also recommend that the current Peruvian TB screening

target be revised. Sputum smear microscopy will give an optimal yield of infectious TB cases and be efficient provided that the operational definition of TB suspects, 2 weeks of cough, is consistently implemented.

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Paper 2

Title: Quality assessment of smear microscopy by stratified lot sampling of treatment follow-up slides

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Summary

Setting

Ten peripheral laboratories performing routine acid-fast bacilli (AFB) smear microscopy in Lima, Peru.

Objectives

To test whether external quality assessment (EQA) rechecking of AFB smears becomes more efficient with stratified lot sampling of treatment follow-up smears.

Design

In 2 consecutive years, a stratified lot sample of 36 treatment follow-up slides and 24 diagnostic slides were randomly selected in each laboratory and blindly rechecked. A second controller determined the final result for discordant slides. Feedback was provided to laboratory technicians during supervisory visits.

Results

More false-negative errors were found in the follow-up slides than in the tuberculosis suspect slides: 25 vs. 3. This represented a yield of 3.5% in 720 follow-up slides and only 0.6% in 480 diagnostic slides. Positive predictive values were high in both years. Respectively three and eight laboratories did not reach a relative sensitivity of >65% during the first and second year, and a clear improvement was seen in only one laboratory. Excessive workload seemed to preclude raising the level of routine performance.

Conclusions

EQA with stratified lot sampling of treatment follow-up slides proved very efficient and effective for identifying laboratories with substandard performance in a setting with low positivity rates in routine diagnostic smears.

Introduction

A strong laboratory network that provides reliable acid-fast bacilli (AFB) smear microscopy services is essential in the control of tuberculosis (TB) [1–3] In areas of high TB prevalence, routine reading of large numbers of slides overloads the scarce human resources, and maintenance of good quality performance becomes a challenge. Older guidelines for quality control recommended rechecking all positive and 10% of negative smears. This was highly inefficient in terms of sample size and composition, wasted efforts in laboratories with high workloads and focused excessively on positive slides. Moreover, the results were often not reliable, as the sample selected was not random, the rechecking not blinded and the final result—the 'gold standard'—relied on one controller only [4].

In 2002, lot quality assurance sampling (LQAS) was recommended for external quality assessment (EQA) of AFB microscopy. LQAS leads to small, feasible sample sizes in large laboratories, especially those with high positivity rates in routine smears [5], and has been successfully applied in different settings [6–8]. However, the sample size required is still too large for countries with decentralised services and moderate-to-low positive prevalence in routine smears, as the numerous small laboratories each require a large sample proportional to their volume of work. This is the case for various countries in South America, and one of the reasons why they continue to apply the old EQA recommendations reporting very low error rates for large numbers of rechecked slides [9].

In this study, we tested a modification to the current EQA guidelines and LQAS. To keep rechecking effective but the sample size small despite a low prevalence of positive slides, the lots were stratified into treatment follow-up and diagnostic slides.

Methods

Setting

The study was conducted between October 2003 and September 2005 in the District of San Juan de Lurigancho in Northeastern Lima, Peru. In 2005, the TB incidence in the district was 213 per 100 000 population [10]. In 2008, the national human immunodeficiency virus prevalence rate in adults and children was estimated at 0.3% [11]. Ten first-level health centre laboratories in the district were selected for the study. Eight were performing only AFB smear microscopy, while the other two were also performing mycobacterial culture. The National Tuberculosis Programme (NTP) guidelines for AFB

microscopy were followed using hot Ziehl-Neelsen (ZN) staining at 0.3% basic fuchsin concentration. Diagnostic smear microscopy was performed on two smears. One to two follow-up smears were examined after each month of the intensive treatment phase. For the study, slide rechecking by a first and second controller was done by two experienced biologists at the Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru.

Study procedures

The sample size for rechecking was determined for the subset of treatment follow-up smears. It was estimated to contain at least 10% positive and scanty positive results. Considering the generally low reproducibility of readings of follow-up smears (mostly 1+ and scanty positive), a minimum relative sensitivity (ability of the laboratory's technician to detect AFB relative to the controllers) of 65% and acceptance number zero (maximum number of false-negative errors allowed in the sample) were considered appropriate. These conditions resulted in a sample size of 36 follow-up smears per year to be rechecked for each laboratory. An arbitrary number of 24 diagnostic smears from TB suspects were added to each laboratory sample to prevent bias during routine reading. The slides were stored in chronological order in the slide boxes rather than based on result.

The laboratory coordinator selected nine follow-up and six suspect smears by stratified random sampling on a quarterly basis. During the second year of the study, positive slides were sampled randomly and added to the lot samples to avoid chance under-sampling of positives, as recommended in the global guidelines [5]. However, contrary to the instructions, the corresponding number of negative slides was randomly removed by mistake.

Selected slides were listed with their identification number, laboratory, and type of sample (follow-up or TB suspect). The coordinator kept the sampling lists with the results obtained by the laboratories. The first controller received the sampled smears with a list showing slide identifiers only. All smears were restained prior to re-reading, which covered 100 fields, as also recommended by national guidelines for routine work. Discordant results between the peripheral laboratories and the first controller were identified by the coordinator. The second controller read the discordant smears, examining as many fields as needed to exclude the presence of AFB with the highest possible probability; reading

was blinded only to the type of discordance, and this result was considered the 'gold standard'. The coordinator compiled all results, identifying the types of errors and their origin (laboratory or first controller). As feedback, and to improve performance, the potential causes of the errors were discussed with the health centre laboratory technicians during the routine supervisory visits of the local laboratory coordinator, and the error slides were reviewed. The definition for the types of errors is provided in the annex at the end of the paper.

Data analysis

All data were entered into an Excel spreadsheet (Microsoft, Redmonds, WA, USA) prepared for the study and double checked. The World Health Organization/ International Union Against Tuberculosis and Lung Disease recommended quantification scale and corresponding classification of major and minor errors were used [5,12].

To eliminate bias due to the considerably different routine prevalence of positives in the different peripheral laboratories and between these and the samples presented to the controllers, relative sensitivities were calculated by applying error rates found in the rechecking sample to the total positive and negative smear results reported in routine work. The relative sensitivity of the first controller's readings was calculated directly from his error rates in the samples rechecked, as explained elsewhere.[13] Furthermore, a ratio of the sensitivity of each laboratory to that of the first controller was calculated, with a value of 1.00 indicating equally sensitive detection by laboratory technicians and controller. Positive predictive values (PPVs) were calculated to provide discriminative power for false-positive error analysis.

Ethical considerations

The study was approved by the Ethics Committee at the Universidad Peruana Cayetano Heredia.

Results

During the 2-year study period, 53 803 routine smears were read at the 10 laboratories (Table 1). The average rate of smear-positive/scanty results for the first and second year was respectively 6.7% and 8.2%, and follow-up smears represented respectively 28.6% and 27.4% of the total examined. A total of 600 slides were selected for

rechecking per year, containing 63 (11%) smear-positive/scanty smears in the first and 182 (30%) in the second year.

Table 1 Numbers of slides examined routinely and numbers rechecked, by laboratory result and year, San Juan de Lurigancho, Lima, Peru

Year, lab n°	Positive		Scanty		Negative		Positive/ scanty in routine %	Positive/ scanty in rechecked sample %
	Routine	EQA	Routine	EQA	Routine	EQA		
October 2003-September 2004								
1	84	4	11	0	1079	56	8	7
2	100	3	32	3	2602	54	5	10
3	221	8	6	0	3131	52	7	13
4	101	1	4	0	1998	59	5	2
5	88	6	13	0	1536	54	6	10
6	124	9	2	1	2058	50	6	17
7	144	9	11	0	1588	51	9	15
8	156	6	18	0	2072	54	8	10
9	377	11	5	0	4008	49	9	18
10	11	2	0	0	813	58	1	3
Total	1406	59	102	4	20885	537	7	11
October 2004-September 2005								
1	63	12	29	0	1799	48	5	20
2	142	16	65	0	2999	44	6	27
3	320	24	46	1	5457	35	6	42
4	153	20	13	0	2769	40	6	33
5	94	14	22	0	2115	46	5	23
6	188	20	9	0	2678	40	7	33
7	158	21	23	0	1969	39	8	35
8	148	23	27	0	1748	37	9	38
9	1032	22	23	0	6206	38	15	37
10	26	9	2	0	1087	51	3	15
Total	2324	181	259	1	28827	418	8	30

EQA=External quality assesment

The numbers of errors detected for TB suspect and treatment follow-up slides are shown in Table 2. The errors of the first controller for the samples from all laboratories taken together appear at the bottom. During the first year, there were three high false-positive (HFP) errors and 11 false-negative errors in the laboratories, of which nine were high false-negative (HFN); during the second year, these fi gures were respectively 2 and 17 (13 HFNs). During the first year, three laboratories were identified with more than one false negative error (mostly HFN), and four (all HFN) during the second year. There was thus no overall improvement in the laboratories during the second year, except for one laboratory (number 5) that went from 3 to 0 HFN. The first controller went from 3 to 0 HFP, but with an apparent deterioration for false-negatives (from 5 to 11, mostly HFN). Low false-positive and quantification errors were rare throughout the study period.

As also shown in Table 2, false-negative errors were far more frequent in the follow-up than in the suspect smears for both the laboratories (10/11 and 15/17 during first and second year) and the first controller (4/5 and 7/11). This represents, for the laboratories, a yield of 25 false-negative errors in 720 follow-up smears (3.5%) against only three such errors in 480 TB suspect smears (0.6%). It is also of note that in the second year at least one false-negative error was detected in the follow-up smears of 8/10 laboratories.

Table 2 Numbers of errors found in the rechecked slides, by type (suspect or follow-up) and year, San Juan de Lurigancho, Lima, Peru

Type of slide, laboratory n°	October 2003 – September 2004					October 2004- September 2005				
	HFP	LFP	HFN	LFN	QE	HFP	LFP	HFN	LFN	QE
Suspect smears										
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	1	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	1	0	0
5	0	0	1	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	1	0	0
7	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	1	0	0	0	0
Total	1	0	1	0	0	1	0	2	0	0
First controller	0	0	1	0	2	0	0	4	0	0
Follow-up										
1	0	0	1	2	0	0	0	1	0	0
2	0	0	4	0	0	0	0	2	1	0
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	3	1	0
5	1	0	2	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	2	0	0
7	0	0	0	0	0	0	0	2	0	1
8	0	0	1	0	0	1	0	1	0	0
9	1	0	0	0	0	0	0	0	1	0
10	0	0	0	0	0	0	0	0	1	0
Total	2	0	8	2	0	1	0	11	4	1
First controller	3	1	3	1	0	0	0	6	1	0

HFP = high false-positive; LFP = low false-positive; HFN = high false-negative; LFN = low false-negative; QE = quantification error.

Table 3 converts the errors to relative sensitivities of the laboratories and to sensitivity ratios with the first controller. Relative sensitivity varied from 16% to 100% for follow-up smears, and from 58% to 100% for suspect smears. Considering suspect and follow-up smears separately, respectively one and three laboratories did not reach 65% relative sensitivity during the first year, vs. respectively two and eight during the second year. Relative sensitivity increased in only one laboratory. The average sensitivity also decreased slightly. The PPV was high for all laboratories in both years. The first controller scored 93% relative sensitivity in both years.

Table 3 Relative sensitivities of laboratories and sensitivity ratio to the first controller by year of study, San Juan de Lurigancho, Lima, Peru

Type of slide, laboratory n°	October 2003 – September 2004			October 2004- September 2005		
	Relative sensitivity %	Sensitivity ratio to first controller	Positive predictive value%	Relative sensitivity %	Sensitivity ratio to first controller	Positive predictive value %
Suspect smears						
1	100	1.07	100	100	1.07	100
2	100	1.07	100	100	1.07	100
3	100	1.07	75	100	1.07	100
4	100	1.07	100	58	0.62	100
5	64	0.69	100	100	1.07	100
6	100	1.07	100	61	0.65	100
7	100	1.07	100	100	1.07	100
8	100	1.07	100	100	1.07	100
9	100	1.07	100	100	1.07	100
10	100	1.07	100	100	1.07	89
Total	94	1.01	96	91	0.98	99
Follow-up smears						
1	25	0.27	100	38	0.41	100
2	27	0.29	100	24	0.25	100
3	100	1.07	100	100	1.07	100
4	100	1.07	100	16	0.17	100
5	31	0.33	80	100	1.07	100
6	100	1.07	100	41	0.44	100
7	100	1.07	100	34	0.36	100
8	71	0.77	100	58	0.62	89
9	100	1.07	80	61	0.65	100
10	100	1.07	100	37	0.40	100
Total	62	0.67	95	43	0.46	98

Discussion

The application of EQA guidelines for AFB smear microscopy modified by stratified lot sampling of treatment follow-up slides was very efficient in screening laboratories for possible substandard performance. Far more false-negative errors were found in the follow-up slides than in the TB suspect slides.

Blinded rechecking of routine smears after LQAS is considered to be the best EQA method [5]. If correctly executed, it yields a realistic view of daily routine performance, allows identification of laboratories with problems that need to be solved and is highly motivating for the laboratory technicians.

Its implementation is feasible in most countries with high TB incidence, but requires an excellent understanding of the procedure and good organisation [14]. However, where the cost of labour is high, the prevalence of positive smears is low and/or services are highly decentralised, its implementation remains problematic. The latter situation is typical in middle-income countries, where numerous laboratories detect a few cases each. In such a setting, sample sizes determined using the LQAS system turn out to be higher than those with the old '10% rule'. Although efficient in terms of detecting errors, the total number of slides to be rechecked becomes prohibitive.

In this study, we tried to circumvent this problem by stratified sampling, targeting treatment follow-up smears. The rationale for using follow-up smears from initially smear-positive cases is to minimise the sample size needed. A considerable proportion of TB patients continue to excrete (dead) bacilli during the first few months of treatment [15]; However, their sputum samples are generally scanty. Reading has lower inherent reproducibility, more errors can be allowed and the target sensitivity can be set lower. Both lower target sensitivity and higher prevalence of positive (and scanty) smears lead to smaller LQAS sample sizes. In all other respects, the rechecking technique applied here followed standard guidelines, including blinding of the first controller and a second controller for discordant slides [5]. Furthermore, we systematically restained before the control reading to also detect staining problems, which are common in situations of work overload, as was suspected in our laboratories.

Positive slides rarely present problems, and if errors occur they are systematic and easily detected with a small sample. They might nevertheless be missed where there

is a low prevalence of positive smears. We therefore oversampled positive slides during the second year. Virtually no false-positives were detected, although three times more had been rechecked. This confirms that very small sample sizes would not hide a false-positive problem. This over-sampling of positives unintentionally resulted in slightly smaller sampling of negatives during the second year; however, the very high number of failing laboratories suggests that even so the statistical power had remained sufficient.

We used fixed sample sizes despite variability in the positivity rate between the laboratories and oversampling of positive slides in the second year. We compensated for this during analysis by applying the error rates detected to the total number of positive and negative slides registered in each laboratory over the year, thus calculating a relative sensitivity compared to the controllers. Likewise, the relative sensitivity for the first controller was calculated by applying his error rate to the total sample rechecked. It remained excellent for both years. This demonstrates that his increased false-negative error rate was explained by the far higher prevalence of positives in the samples rechecked during the second year and not by decreasing performance. In general, conversion to relative sensitivity can be recommended for identifying deficiencies [13].

Follow-up smears were targeted because of their higher positive prevalence to reduce the necessary sample sizes, but also because they are more difficult to read correctly and a good indicator of quality in AFB microscopy laboratories [16]. It could be argued that sampling these smears was not fair and caused a distorted view of the laboratory's real performance. The large majority of false-negative errors were indeed found in the follow-up smears, and it is likely that the laboratories' overall performance is better than suggested by our results (or, alternatively, that they neglect follow-up smears). Notwithstanding, the relative sensitivity ratio still indicates large deficiencies in many laboratories compared to a controller with a good microscope and good technique who follows the guidelines. For a rough but more objective view of case detection errors, calculations should include TB suspect smears only, but this is not really the point. In some of our laboratories, overall relative sensitivity did not even reach 50%, indicating that more than half of the positive smears had been missed, at least among follow-up smears. Our approach differentiates performance more clearly than a simple application of the pass/no pass rule that comes with the standard LQAS method, and the laboratories most in need of problem-solving supervision are more clearly identified.

In 2004, 152,577 AFB smears were rechecked in Peru (all positives and 10% negatives), and 99.7% agreement was observed [9]. It could be argued that the discordance rate was within the normal range of variation of the smear microscopy technique. However, our results, and those of other authors [17,18] indicate that the rechecking system in place is not capable of identifying the problems that need to be addressed.

While our approach manifestly succeeded in efficiently identifying laboratories with unsatisfactory performance, the lack of improvement during the second year—in all but one laboratory—indicates a failure of follow-up and remedial action. The study included regular feedback and supervision, with error review and the occasional repair of a microscope. However, the excessive workload caused by tuberculosis programme policy could not be reduced. The NTP sets a performance target of TB suspects to be screened monthly. To reach this target, patients with < 2 weeks of cough are screened, which leads to low smear positivity rates, overburdened laboratories and, as shown here, poor performance despite EQA and corrective measures [19].

In line with current recommendations, NTPs should emphasise quality and not quantity of sputum smear examinations [20,21]. The introduction of methods that improve efficiency, such as the LED fluorescence microscopy [22,23], could be worthwhile in the most overloaded laboratories. Finally, rechecking of routine slides should be made more efficient and effective by reducing sample size and ensuring excellent technical execution. Stratified LQAS of treatment follow-up smears permits efficient EQA of AFB smear microscopy in decentralised settings with a low prevalence of positives in laboratory routine, as in Peru.

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Annex. Definition of errors for the External Quality Assesment for Smear Microscopy (1).

- **Major error**: This type of error is considered the most critical since it has the highest potential impact on patient management, and can result in an incorrect diagnosis or improper management of a patient. Major errors may indicate gross technical deficiencies, and include both High False Positive and High False Negative errors.

    - **High False Positive** (HFP): A negative smear that is misread as 1+ to 3+ positive. This is a major error.

    - **High False Negative** (HFN): A 1+ to 3+ positive smear that is misread as negative. This is a major error.

- **Minor error**: In clinical practice, these errors may have some impact on patient management. However, for the purpose of evaluating laboratory performance, this type of error is considered less serious, because of inherent limitations in consistently detecting a few AFB that may be unequally distributed within a smear. The frequency of minor errors may indicate technical deficiencies.

    - **Low False Positive** (LFP): A negative smear that is misread as a low (1-9AFB/100fields) positive. This type of minor error occurs occasionally even in laboratories that are performing well.

    - **Low False Negative** (LFN): A low (1- 9AFB/100fields) positive smear that is misread as negative. This type of minor error occurs occasionally even in laboratories that are performing well.

- **Quantification Error** (QE): Difference of more than one grade in reading a positive slide between examinee and controller. This is a minor error that generally has no impact on case management.

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Paper 3

Title: A prospective longitudinal study of tuberculosis among household contacts of smear positive tuberculosis cases in Lima, Peru

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Abstract

Background

Household contacts (HHCs) of TB cases are at increased risk for TB disease compared to the general population but the risk may be modified by individual or household factors. We conducted a study to determine incident TB among HHCs over two years after exposure and to identify individual and household level risk factors.

Methods

Adults newly diagnosed with a first episode of smear-positive pulmonary TB (index cases) between March 2010 and December 2011 in eastern Lima, were interviewed to identify their HHC and household characteristics. TB registers were reviewed for up to two years after the index case diagnosis and house visits were made to ascertain TB cases among HHC. The TB incidence rate ratio among HHCs as a function of risk factors was determined using generalized linear mixed models.

Results

The 1178 index cases reported 5466 HHCs. In 402/1178 (34.1%) households, at least one HHC had experienced a TB episode ever. The TB incidence among HHCs was 1918 (95%CI 1669-2194) per 100,000 person-years overall, and was 2392 (95%CI 2005-2833) and 1435 (95%CI 1139-1787) per 100,000 person-years in the first and second year, respectively. Incident TB occurred more than six months following the index case's TB diagnosis in 121/205 (59.0%) HHCs. In HHCs, bacillary load and time between symptoms and treatment initiation in the index case, as well as the relationship to the index case and the sex of the HHC all had a significant association with TB incidence in HHCs.

Conclusions

Incidence of TB among HHCs was more than ten times higher than in the general population. Since certain HHC and households were at higher risk of TB, we recommend studies comparing HHC investigation to households at highest risk versus current practice, in terms of efficiency.

Background

Household contacts (HHCs) of tuberculosis (TB) patients are at higher risk for TB infection and disease. In recent studies, 4.5% to 7.8% of HHCs have been found to have active disease [1-3]. Investigating HHCs may facilitate timely diagnosis and treatment of active TB as well as of latent TB infection. However, contact tracing in general and HHC investigation in particular are resource-intensive activities, and their consistent implementation is a challenge in both high- and low-income countries. TB control programs in low-income high-incidence settings face competing priorities[4-6] but limited data suggest that screening HHCs of TB cases for active disease may be cost-effective in low- and middle-income countries[7]. The World Health Organization recently extended its recommendations for HHC tracing to these countries[8].

TB risk in HHCs may vary by individual and household as well as by background community incidence, and investigating all HHCs in high-burden, low-resource countries may be inefficient or unfeasible. Estimates of risk should consider the correlation of risk factors of HHCs within a single household[9] to avoid overestimating effects when ignoring that correlation. To better inform TB programs on the precise burden of TB over time in HHCs, studies with sufficiently long follow-up and with comparable methods need to be conducted[1,2,10]. A better understanding of TB risk over time and risk factors would provide evidence to increase efficiency of HHC investigation guidelines in high TB burden settings[6] by strategies and timing following epidemiological data.

Studies have shown a high burden of TB among HHC of multidrug resistant (MDR) TB and drug susceptible cases in Peru[10-13]. We conducted a prospective study to determine the TB incidence over 2 years among HHC of all new TB cases in eastern Lima, and to identify individual and household level risk factors that determine this incidence.

Methods

Design, setting and study population

We conducted a longitudinal prospective study of TB incidence among HHCs of smear-positive cases in an eastern Lima district. The district is the most populous in Peru with 1,047,026 inhabitants in 2013 of which 27% are living in poverty, higher than the 17.5% for Lima[14]. TB incidence in the district in 2012 was 175 cases of TB per 100,000 inhabitants, and among those patients tested for HIV 2.9% were positive. The district has

34 public health facilities where clinics implementing the directly observed treatment short-course strategy (DOTS) operate. Consenting adults diagnosed with a first episode of smear-positive pulmonary TB between March 2010 and December 2011 (index cases) were enrolled in the present study.

Peruvian guidelines recommend evaluation of HHCs (symptoms screening, tuberculin skin test and chest X-ray) at the time of diagnosis of the index case, and at the second and sixth month of the index case treatment. Isoniazid prophylaxis for TB is recommended to all HHCs under five years old and to HHC from 5 to 19 years old with latent tuberculosis infection documented with a positive tuberculin skin test. The present study did not interfere with routine HHC investigation by National TB Program health center staff. In 2010-2011, the study district reported that 74% of HHCs had been evaluated and that prophylaxis had been prescribed to 54% of eligible HHCs.

Baseline data collection and longitudinal detection of TB among HHCs

At enrollment, trained research field workers interviewed index cases to collect demographic and HHC data using a structured questionnaire. Index cases provided the names of their HHCs and any previous TB episodes among them. To determine incident TB for known HHCs, district TB registers were reviewed periodically for up to two years after the diagnosis of the index case. In each of these reviews, the names of newly registered TB cases were compared to the names of the HHC reported by index cases to capture TB diagnoses among HHCs. At the end of the study, the database of HHC reported by the index cases was matched with the TB registers in order to capture HHC registered as a TB case but missed by the periodic visual inspection. Queries were run to match combinations of given and family names and initials, thus reducing the risk of missing matches. To confirm true matches, two authors (LO and KV) subsequently verified all hits.

To capture TB diagnoses outside the study district or in a private health facility, a single household visit was conducted at the end of the two-year follow-up period. During that visit, the index case or the head of the household was asked if any of the HHC had developed TB since the index case diagnosis. Households where the index case had defaulted treatment or died did not receive the final follow-up visits. This was the case for 12.8% (151/1178) of the study households.

Study definitions

A HHC was defined as a person sleeping under the same roof and sharing cooking facilities with the index case for at least three months before the case's diagnosis. Past TB in an HHC was defined as a TB episode occurring more than two years before the diagnosis of the index case; recent TB was defined as TB diagnosed within the two years before the diagnosis in the index case. Incident TB in an HHC was defined as TB that came to diagnosis up to two years following the diagnosis of the index case.

Analysis

Data were entered in an Access (Microsoft Redmond, WA, USA) database and analyzed with Stata v.12 (Stata Corp, 12.0, College Station, TX).

Index cases who lived in congregate settings such as shelters or rehabilitation centers for addicts and those who lived alone were excluded from the analysis. The contact's, household and index case's characteristics of HHC with incident TB were compared to those of HHC without an episode of TB. As our data source for incident TB episodes among HHC was the district TB register, which was checked up to two years after the inclusion of the last index case, complemented with a final household visit, we considered the two years follow up complete for all HHC. Only those that developed incident TB were censored.

Risk factors for acquiring TB, including those not measured directly, may be correlated at household/index case level. To account for this correlation, we used generalized linear mixed models (GLMM) with random effects for household, in Poisson regression analysis for count outcomes. A simple logistic regression model may overestimate the effects measured. The outcome was the number of HHCs with incident TB. To protect against the impact of model-misspecification, robust standard errors were used. Covariates that were significant (at $p<0.2$) in the bivariate analysis as well as those that were a priori expected to have an influence on or to be confounding the incidence of TB in HHC were considered for inclusion in the model. Models were compared by backward selection. Variables with the weakest association were taken out until a significant difference between two models was found.

Ethical considerations

The protocol was approved by the Institutional Ethics Committees from the Universidad Peruana Cayetano Heredia and from the University of Antwerp. All enrolled index cases provided written informed consent. The data was analyzed anonymously.

Results

Study population

The study enrolled 1295 participants with a first diagnosis of smear-positive TB. Of these, 117 (9.0%) were excluded: 30 were HHCs of a previously enrolled case, 59 lived alone and 28 lived in congregate settings. Included and excluded index cases were similar in age, but a higher proportion of excluded participants were male (81.0% *vs.* 59.2%) ($p<0.001$). The 1,178 (91%) index cases included in the analysis reported 5,466 HHCs (median number per index case = 4, interquartile range (IQR) 3-6). Table 1 shows the characteristics of the index cases, of the household and of the HHCs.

TB burden in households and among HHCs

Of the HHCs, 602 (11.0%) HHC had experienced at least one TB episode –either past, recent or incident. Thirty (0.6%) HHC had more than one episode. Past TB was reported for 292 (5.3%) HHCs, recent TB for 135 (2.5%) HHC, 205 (3.8%) had incident TB. The median time between the diagnosis of a recent TB episode in a HHC and the diagnosis of the index case was 275 days (IQR, 86-483). Table 2 shows the burden of TB among HHCs per household: in 402/1178 (34.1%) households, at least one HHC had a TB episode at some point and, in 163 (13.8%) households, at least one incident TB episode occurred during the follow up period. Incident TB episodes occurred among 3.8% of HHC, which corresponds to 1918 (95%CI 1669-2194) incident TB episodes per 100,000 person-years. The TB incidence rate among HHCs was 2392 (95%CI 2005-2833) per 100,000 person-years in the first year and 1435 (95%CI 1139-1787) per 100,000 person-years in the second year. The median time between the index case diagnosis and incident TB episode was 256 days (8.4 months, IQR, 94-455). Of the 673 HHCs ≤ 5 years old, 16 (2.4%) developed incident TB. Figure 1 shows the cumulative number of incident TB; 121/205 diagnoses (59.0%) occurred beyond the sixth months following the index cases' enrollment.

Table 1 Characteristics of the index cases and household contacts

Characteristics		N (%) *
Index case characteristics (n=1178)		
Male sex		481 (40.0)
Age (median, IQR) in years		26 (21-35)
Presence of cough		1135 (96.4)
Time from symptoms to treatment initiation (in days)		33 (19-62)
Smear positivity		
	Scanty	40 (3.4)
	+/++	562 (47.7)
	+++	576 (48.9)
Treatment		
	For drug susceptible TB	1162 (98.6)
	For MDR-TB	16 (1.4)
Tobacco use		
	Never	713 (60.7)
Smokes		416 (35.4)
Used to smoke		46 (3.9)
HIV		
	Negative	884 (75.0)
	Positive	18 (1.5)
	Not tested	276 (23.4)
Household characteristics		
Past TB episodes		62 (5.3)
Recent TB episodes		29 (2.5)
Persons per room		2.1 (1.5-3.0)
Household contact characteristics (n=5466)		
Male sex		2674 (48.9)
Age (median, IQR) in years		24 (12-40)
Relationship with the index case		
	Sibling	1416 (25.9)
	Parent	946 (17.3)
	Partner	437 (7.9)
	Offspring	1031 (19.9)
	Other	1636 (29.9)

* frequencies are presented in absolute number and percentages, except for continuous variables where the median and the interquartile range (IQR) is presented. TB = tuberculosis, MDR-TB = multi drug resistant TB. For smear positivity: scanty = 1-9 acid-fast bacilli (AFB) in 100 fields, +/++ = from 10 AFB in 100 fields to 10 AFB per field in at least 50 fields, +++ = more than 10 acid fast bacilli in at least 20 fields. Past TB = a TB episode in a HHC more than two years before the diagnosis of the index case; recent TB = a TB episode within two years before the index case diagnosis.

Household contacts characteristics associated to incident TB in HHC

At individual contact level, relationship to the index case, sex and age were associated with incident TB in HHCs in bivariate analysis (Table 3). To determine if young

children of mothers with TB had a higher risk of TB, we looked for an interaction of the age of the HHC and the index case being a mother or not, but found none.

Table 2: Past, recent and incident tuberculosis episodes among household contacts, per household. Lima, Peru, 2010-2013

Number of tuberculosis episodes in household	N° Households	% Households
≥1 past case only	149	12.6
≥1 recent case only	64	5.4
≥1 incident case only	105	8.9
≥1 past + ≥1 recent case	26	2.2
≥1 past + ≥1 incident case	35	3.0
≥1 recent + ≥1 incident case	17	1.4
≥1 past + ≥1 recent + ≥ 1 incident case	6	0.5
No cases	776	65.9
Total	1178	100

Past case = household contact that had a tuberculosis episode more than two years before the diagnosis of the index case. Recent case = household contact that had a tuberculosis episode in the two years previous to the diagnosis of the index case. Incident TB case = TB episode in a household contact after the diagnosis of the index case.

Index case and household characteristics associated to incident TB in HHC

Bivariate analysis for household and index case risk factors is shown in Table 4 (1178 households). Time from symptoms to treatment in the index case, sputum bacillary load and a past TB episode in any member of the household were associated with incident TB in HHCs. Tobacco use, education, working status and marital status of the index case and socio-economic level of the household were not associated with incident TB (data not shown).

Multilevel analysis of index case, household and household contacts characteristics associated to incident TB in HHC

Table 5 shows the results of the multivariate analysis and the supplementary table shows the bivariate analysis of additional characteristics. Bacillary load and the time from symptoms to treatment of the index case, as well as the relationship with the index case and the sex of the HHC remained significantly associated with incident TB in HHCs. No household characteristics studied were significantly associated with incident TB. The standard deviation of the between-household difference on the effect of the incidence rate ratio of TB was 1.4, 95%CI 1.1-1.9. This finding suggests that, in addition to the

characteristics studied, unmeasured covariates specific to each index case/household also affect TB incidence in HHCs.

Table 3: Incident tuberculosis in 5466 household contacts (HHC) in function of the HHC's characteristics. Lima, Peru, 2010-2013.

	Incident TB in the HHC		Crude odds ratio (95%CI)
	Yes	No	
Age in years			
≤ 5	16 (2.4%)	657 (97.6%)	1.0 (0.5-1.8)
6-19	56 (3.8%)	1407 (96.2%)	1.5 (1.0-2.5)
20-40	100 (5.0%)	1883 (95.0%)	2.1 (1.4-3.2)
≥41	33 (2.5%)	1314 (97.6%)	1
Sex			
Female	88 (3.2%)	2704 (96.9%)	1
Male	117 (4.4%)	2557 (95.6%)	1.4 (1.1-1.9)
Relationship			
Sibling	84 (5.9%)	1332 (94.1%)	2.9 (2.0-4.4)
Parent	21 (2.2%)	925 (97.8%)	1.0 (0.6-1.8)
Partner	27 (6.2%)	410 (93.8%)	3.0 (1.8-5.0)
Offspring	38 (3.7%)	993 (96.3%)	1.8 (1.1-2.8)
Other	35 (2.1%)	1601 (97.9%)	1
Past TB			
Yes	14 (4.8%)	278 (95.2%)	1.3 (0.7-2.3)
No	191 (3.7%)	4983 (96.3%)	1
Recent TB			
Yes	6 (4.4%)	129 (95.6%)	1.2 (0.5-2.7)
No	199 (3.7%)	5132 (96.3%)	1

HHC=household contact

Among the 287/5466 HHCs who had three of the above determinants –excluding male sex- associated to incident TB (siblings/partners, index case with a time from symptoms to treatment of > 30 days and a high bacillary load), 29 (10.1%) had incident TB; among the 2419 HHC that had two of those factors, 159 (6.6%) had incident TB.

Discussion

The incidence of TB in HHC of new smear-positive TB cases in this district was 1918 per 100,000 person-years, ten times higher than that for the general population in the district. Among incident TB cases, 59% occurred beyond six months of the index case's diagnosis and would not have been identified as by current HHC investigation contracting procedures. TB burden in households was high, with 34% of households having a HHC with past, recent or incident TB in addition to the index case. Being a sibling or a partner

of the index case, and more than 30 days between symptoms treatment initiation or a high bacillary load in the index cases were associated with incident TB.

Table 4. Incident tuberculosis among household contacts in the 1178 households, in function of the index case and household characteristics. Lima, Peru, 2010-2013.

	Incident TB in the household		Crude odds ratio (95%CI)
	Yes	No	
Index case characteristics			
Sex			
Female	86 (12.3%)	611 (87.7%)	0.7 (0.5-1.0)
Male	77 (16.0%)	404 (84.0%)	1
Age			
18-27	96 (14.4%)	571 (85.6%)	1.0 (0.6-1.5)
28-37	34 (14.2%)	206 (85.8%)	0.7 (0.4-1.4)
38-47	11 (11.0%)	89 (89.0%)	0.9 (0.5-1.4)
>48	22 (13.8%)	149 (87.1%)	1
Cough			
Yes	160 (14.1%)	975 (85.9%)	1
No	3 (7.0%)	40 (93.0%)	0.5 (0.1-1.5)
Time from symptoms to treatment [a]			
0-30 days	50 (10.0%)	448 (90.0%)	1
>30 days	113 (16.6%)	567 (83.4%)	1.8 (1.3-2.5)
Smear positivity at diagnosis [b]			
Scanty	1 (2.5%)	39 (97.5%)	0.2 (0.0-1.3)
+/++	110 (12.7%)	755 (87.3%)	1
+++	52 (19.1%)	221 (80.9%)	1.6 (1.1-2.3)
Treatment type			
Drug sensitive	159 (13.7%)	1003 (86.3%)	0.5 (0.2-1.5)
Multidrug resistant	4 (25.0%)	12 (75.0%)	1
Household characteristics			
Past TB episodes [c] in the household			
Yes	41 (19.0%)	175 (81.0%)	1.6 (1.1-2.4)
No	122 (12.7%)	840 (87.3%)	1
Recent TB episodes [d] in the household			
Yes	23 (20.4%)	90 (79.7%)	1.7 (1.0-2.8)
No	140 (13.2%)	925 (86.9%)	1
Persons per room (median, IQR)	2.5 (1.8-3.8)	2 (1.5-3)	1.1 (1.0-1.2)

[a] = time from symptoms was calculated from the date the participant reported to start coughing until the date of treatment registered, [b]scanty = 1-9 acid fast bacilli (AFB) in 100 fields, +/++ = from 10 AFB in 100 fields to 10 AFB per field in at least 50 fields, +++ = more than 10 acid fast bacilli in at least 20 fields,smear positivity was classified following standard national TB program guidelines[13], [c] = a TB episode in a HHC more than two years before the diagnosis of the index case, [d] = a TB episode within two years before the index case diagnosis.

Table 5: Index case, household and household contacts characteristics and their relation to incident TB among household contacts within two years of the index case´s diagnosis.

		Bivariate GLMM		Multivariate GLMM	
		IRR	95%CI	IRR	95%CI
Index case characteristics					
Sex					
	Female	1			
	Male	0.7	0.5-1.0	-	
Age					
	18-27	1.3	0.8-2.3	-	
	28-37	1.6	0.8-2.9	-	
	38-47	1.1	0.5-2.6	-	
	>48	1			
Cough					
	Yes	1			
	No	0.5	0.1-1.8	-	
Time from symptoms to treatment [a]					
	0-30 days	1		1	
	>30 days	1.9	1.3-2.8	1.8	1.2-2.7
Smear positivity at diagnosis [b]					
	Scanty	0.2	0.02-1.4	0.2	0.03-1.7
	+/++	1		1	
	+++	1.6	1.1-2.4	1.5	1.02-2.3
Treatment type					
	Drug sensitive	1			
	Multidrug resistant	1.6	0.5-5.4	-	
Household characteristics					
Past TB episodes [c] in the household					
	Yes	1.2	0.6-2.1	-	
	No	1			
Recent TB episodes [d] in the household					
	Yes	1.2	0.5-2.8	-	
	No				
Persons per room		1.01	0.9-1.1	-	
Household contact characteristics					
Sex					
	Female	1		1	
	Male	1.5	1.1-1.9	1.4	1.1-1.9
Age					
	0-5	1.0	0.5-1.8	0.9	0.4-1.9
	6-19	1.7	1.1-2.6	1.2	0.7-2.3
	20-40	2.3	1.5-3.5	1.5	0.9-2.6
	>40	1		1	
Relationship					
	Sibling	2.8	1.8-4.3	2.6	1.6-4.0
	Parent	0.9	0.5-1.6	1.1	0.5-2.3
	Partner	2.9	1.7-5.0	2.7	1.5-4.8
	Offspring	1.5	0.9-2.5	1.5	0.9-2.4
	Other	1		1	

GLMM: generalized linear mixed models, IRR: incidence rate ratio, CI: confidence interval. [a] = time from symptoms was calculated from the date the participant reported to start coughing until the date of treatment registered, [b]scanty = 1-9 acid fast bacilli (AFB) in 100 fields, +/++ = from 10 AFB in 100 fields to 10 AFB per field in at least 50 fields, +++ = more than 10 acid fast bacilli in at least 20 fields, [c] = a TB episode in a HHC more than two years before the diagnosis of the index case, [d] = a TB episode within the two year

Supplementary table: Index case characteristics – additional to those presented in table 5- and their relation to incident TB among household contacts within two years of the index case´s diagnosis.

		Bivariate GLMM analysis	
		IRR	95%CI
Working status			
	Student	1	
	Working	1.5	0.7-3.4
	Unemployed	1.9	0.9-3.9
Marital status			
	Single	1	
	Married/cohabiting	0.8	0.5-1.6
	Divorced/widowed	1.1	0.6-2.0
Tobacco use			
	Never	1	
	Used to smoke / smokes	0.6	0.3-1.3
Diabetes mellitus			
	No	1	
	Yes	0.7	0.3-1.6
Education level [a]			
	Primary	1	
	High school	0.9	0.6-1.2
	Higher education	0.9	0.6-1.4
HIV			
	Negative	1	
	Positive	0.5	0.1-2.2
	Not done	0.7	0.4-1.2
Body mass index at diagnosis		1.0	0.9-1.1
Socio economic status			
	Not poor	1	
	Poor	1.5	0.9-2.2

GLMM: generalized linear mixed models, IRR: incidence rate ratio, CI: confidence interval.
[a] = highest level achieved

Index cases included in the study were representative of the new TB cases in the district, but this study has some limitations. Incident TB in HHC could have been diagnosed outside the district or in facilities not managed by the Ministry of Health and not appear in the TB register. We conducted household visits to ascertain these cases. However, households of index cases who defaulted treatment or died were not visited.

This could lead to underestimating TB incidence in HHC. Furthermore, our definition of incidence was based on diagnosis and case notification. Since only individuals with symptoms are likely to present for diagnosis, true incidence may be underestimated. Past and recent TB episodes might also be underestimated as these were reported by the index case. Strengths of this study include its prospective design, the use of two complementary follow up approaches to ascertain incident TB in HHC and accounting for the correlation of data at household level in the analysis.

In a systematic review of 25 studies in low- and middle-income countries, the weighted estimated average of incident TB in HHC in the first year of follow-up was 478 (95%CI 897-2427) per 100,000 person-years and 831 (95%CI 624-1106) per 100,000 person-years in the second year[1]. We found rates approximately two and five times higher, respectively. Some studies conducted in Lima have determined incident TB among HHCs of selected TB cases. In the first and in the second year, 2360 incident TB episodes per 100,000 person-years occurred among HHC of 80 MDR-TB index cases in southern Lima[10], while a more recent study found 2456 incident TB episodes per 100,000 person-years occurred among HHC of 213 multidrug resistant TB cases and 4351 incident TB episodes per 100,000 person-years among HHC of 487 drug susceptible index cases[13]. A retrospective study that included 693 MDR and extensively drug resistant TB index cases found 3165 and 1092 incident TB cases in HHC per 100,000 person-years in the first and second year, respectively[12]. Our data demonstrate similar high rates of incident TB among HHCs of a large sample of unselected new pulmonary TB patients.

Our findings confirm that siblings and partners[15,16], male HHC[10,16], and HHC exposed to index cases with higher sputum bacillary loads[13] or that are infectious for a longer period[17] are more likely to develop incident TB. Contrary to what is expected, HHC under six years old were not found to be at higher risk for incident TB than older age groups. However, it is possible that TB in children is under diagnosed in Peru. One explanation consistent with our findings is that there are important effects at the household/index case level on the incidence of TB in HHCs. This was also found in a multilevel study in Pakistan that looked into household and HHC determinants of TB infection[9]. Characteristics at household level that may have influence should be considered in future studies, such as ventilation, sleeping arrangements, frequent exposure to non-household members as well as genetic predisposition of families. Measuring these potential risk factors might strengthen the index case/household

association and incidence rate ratio of TB among HHCs. Prioritizing individuals at highest risk for HHC investigation could reduce the number of HHC to be screened, but such selection may be logistically cumbersome. In addition, staff, patients and HHC themselves may not find it acceptable. However, it could be an option to use index case characteristics that put households at higher risk in order to select complete households in which to screen all contacts.

While HHCs of active TB patients may have a much higher risk of TB than the general population, TB cases in HHCs still account for only a small proportion of the total TB burden in a medium and high incidence area[18]. For example, the 205 incident cases found in our study corresponded to approximately 5% of the district's TB notifications during the same period. Furthermore, in some high incidence areas, community transmission can be higher than household transmission[19,20]. The background TB incidence at which full HHC contact investigation becomes cost-effective as compared to investigating only selected high-risk HHC or index cases/households would provide valuable information for National TB Programs decision-making.

Finally, National TB Programs typically attempt to evaluate HHC at the start of the index case's TB treatment, at the change of their treatment phase and at the end of their treatment. However, this approach does not take into consideration the time that may elapse occur between TB infection and disease[1,18,20]. For that reason, even full compliance to Peruvian guidelines for HHC investigation of drug sensitive cases would have failed to identify 59% of incident TB episodes as HHCs.

Conclusions

This study demonstrated that HHC of TB cases in Lima, Peru are at very high risk for incident TB as compared to the general population. Studies comparing the feasibility and cost effectiveness of all-inclusive versus risk based HHC investigation strategies within National TB Programs are needed to inform policy formulation.

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Paper 4

Title: A diagnostic accuracy study of Xpert®MTB/RIF in HIV positive patients with high clinical suspicion of pulmonary tuberculosis in Lima, Peru

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Abstract

Background

Diagnosis of pulmonary tuberculosis (TB) among human immunodeficiency virus (HIV) patients remains complex and demands easy to perform and accurate tests. Xpert®MTB/RIF (MTB/RIF) is a molecular TB diagnostic test which is rapid and convenient; the test requires minimal human resources and reports results within two hours. The majority of performance studies of MTB/RIF have been performed in high HIV burden settings. However, TB diagnostic studies among HIV patients in low HIV prevalence settings such as Peru are still needed.

Methodology/Principal Findings

From April 2010 to May 2011, HIV-positive patients with high clinical suspicion of TB were enrolled from two tertiary hospitals in Lima, Peru. Detection of TB by MTB/RIF was compared to a composite reference standard Löwenstein-Jensen (LJ) and liquid culture. Detection of rifampicin resistance was compared to the LJ proportion method. We included 131 patients, the median CD4 cell count was 154.5 cells/mm3 and 45 (34.4%) had TB. For TB detection among HIV patients, sensitivity of MTB/RIF was 97.8% (95% CI 88.4 99.6) (44/45); specificity was 97.7% (95% CI 91.9–99.4) (84/86); the positive predictive value was 95.7% (95% CI 85.5–98.8) (44/46); and the negative predictive value, 98.8% (95% CI 93.6–99.8) (84/85). MTB/RIF detected 13/14 smear-negative TB cases, outperforming smear microscopy [97.8% (44/45) vs. 68.9% (31/45); p = 0.0002]. For rifampicin resistance detection, sensitivity of MTB/RIF was 100% (95% CI 61.0–100.0) (6/6); specificity was 91.0% (95% CI 76.4–96.9) (30/33); the positive predictive value was 66.7% (95% CI 35.4–87.9) (6/9); and the negative predictive value was 100% (95% CI 88.7 –100.0) (30/30).

Conclusions/Significance

In HIV patients in our population with a high clinical suspicion of TB, MTB/RIF performed well for TB diagnosis and outperformed smear microscopy.

Introduction

Tuberculosis (TB) is the leading cause of death in human immunodeficiency virus (HIV) infected patients [1]. New TB diagnostic tests and strategies are urgently needed within this population. Several new TB diagnostic tests have recently been developed; however, those require further evaluation among HIV infected patients. Ideally these new diagnostic tests should be accurate, provide results in a time frame that allows efficient treatment decision-making without increasing the demand of the already scarce human resources available in countries affected by HIV and TB.

Achieving accurate diagnosis of TB disease is more complex in HIV patients than in subjects with normal immunity [2]. Sputum smear microscopy has limited accuracy amongst HIV infected patients, further complicated by the multiple clinical, subclinical and atypical presentations observed among these patients [2,3]. Furthermore, TB disease can disseminate rapidly in patients with advanced immunosuppression. Prompt diagnosis of TB in HIV patients could lead to early treatment initiation and could contribute to decrease TB-related mortality.

Smear microscopy is the cornerstone of TB diagnosis and case detection in the vast majority of TB control programs [4]. It is inexpensive, has few technical requirements and in settings with high burden of disease, smear microscopy has a high positive predictive value despite its variable (35 to 80%) sensitivity [5]. Nevertheless, conditions such as high HIV rates, concurrent nontuberculosis mycobacterial infections, and multidrug resistant tuberculosis (MDR-TB) can impact its diagnostic yield and effectiveness [6,7]. The current reference standard for TB diagnosis is the culture of Mycobacterium tuberculosis (Mtb).

Culture does allow for drug resistance testing; however the test requires proper laboratory infrastructure and trained personnel and the time required for culture growth is long [8].

Xpert®MTB/RIF (Cepheid, Sunnyvale, USA) is a semiquantitative molecular test for simultaneous detection of TB and rifampicin resistance through detection of the rpoB gen. This test works with the GeneXpert® System device (Cepheid, Sunnyvale, USA) that fully automates a real-time polymerase chain reaction (rt-PCR) and provides results within two hours. It has minimal biosafety requirements and reduced technical manipulation [9].

Xpert®MTB/RIF was endorsed in 2010 by the World Health Organization (WHO) for the screening of TB in persons suspected of having MDR-TB or HIV-TB co-infection.

To date, most studies have evaluated the performance of Xpert®MTB/RIF test (from now on referred as MTB/RIF) in pulmonary and extrapulmonary specimens mostly in HIV endemic countries in Africa where up to 80% of TB patients are HIV co-infected [10–17]. We evaluated the performance of MTB/RIF in HIV-positive adult patients with high clinical suspicion of pulmonary TB in two sites in Lima, a setting that has one of the highest TB and MDR-TB rates in the Americas, as well as low (,3%) HIV prevalence in the general population.

Methods

Study Setting

We conducted a cross-sectional study to evaluate the diagnostic test accuracy of MTB/RIF in identifying pulmonary TB disease in HIV patients in two tertiary hospitals: Hospital Nacional Hipólito Unánue (HNHU) in Eastern Lima and Instituto de Medicina Tropical Alexander von Humboldt (IMTAvH) in Northern Lima. In 2010, the incidence for all TB cases in Peru was 110 per 100,000 population and 2.6% were co-infected with HIV [18].

Study Patients

We included patients 18 years of age or older with an HIV diagnosis confirmed by Western Blot, a high clinical suspicion of TB and who had not received more than two doses of TB treatment. A high clinical suspicion of TB was defined as cough for ten or more days with concurrent abnormal chest x-ray (cavity, focal opacity, pleural effusion, nodule or lymphadenopathy) and at least one of the following symptoms: fever, fatigue, night sweats, hemoptysis, chest pain or weight loss. We included those who agreed to participate and completed the written informed consent. Patients who did not provide a second sputum sample with the required volume were subsequently excluded.

Study Procedures

Trained study health personnel interviewed and enrolled study patients using a structured questionnaire for demographic, clinical and epidemiological data. Interviews were conducted prior to obtaining the first sputum sample. Clinical records were reviewed

in case of discrepancies between the reference standard and the MTB/RIF.

Sample Collection and Processing

The microbiology laboratory at IMTAvH conducted diagnostic tests requested by the Peruvian National TB Program [smear microscopy, Löwenstein-Jensen culture (LJ), and LJ proportion method (LJ PM)] and Mycobacteria Growth Indicator Tube (MGIT). Routine tests done on the first sputum sample, usually an on-the-spot sample, were not included in the primary analysis. The following day, the second sample, usually a morning sample, was collected and used to perform direct MTB/RIF and to repeat all the tests done to the first sample. Sputum samples that could not be processed on the same day were stored at 4°C and processed the following morning or on Monday if it was collected on a Saturday.

The study staff transported all the samples by car on a daily basis at 4°C from HNHU to the microbiology laboratory at IMTAvH (distance ~ 20 minutes). All tests were performed according to standard protocols and established guidelines [19,20]. Briefly, 3 ml of sputum were transferred to a 50 ml Falcon tube to be decontaminated with N-acetyl-L-cysteine and sodium hydroxide; of the decontaminated pellet, ~ 0.5 ml was used for smear staining with Ziehl-Neelsen. Two slopes of LJ culture were inoculated with ~0.2 ml sputum pellets. For MGIT, ~0.5 ml sputum pellets were inoculated on liquid medium BD BBL Manual MGIT™ (Cockeysville, MD, USA) and MGIT tubes were read using the BD BACTEC™ MicroMGIT Fluorescence Reader (Cockeysville, MD, USA). Drug susceptibility testing was performed using the LJ PM. For direct MTB/RIF, the sputum sample was carefully mixed to make it homogeneous, then sample reagent was added to 1 ml of untreated sputum on a 2:1 ratio, mixed twice manually during the incubation period for 15 minutes at room temperature, and then 2 ml were transferred to the MTB/RIF cartridge as previously described [21]. The cartridge was closed and placed into the GeneXpert® System for analysis.

Three trained laboratory technicians performed routine tests and the MTB/RIF test was performed by a single technician with experience in handling the GeneXpert® System. All technicians remained blinded to results of the tests they did not perform.

Due to test manufacturer modifications to MTB/RIF software during study performance, we worked with two different MTB/RIF software versions: 2.1 and 4.0. The

new version (4.0) had higher cutoff values for rifampicin detection and did not include changes for Mtb detection (16). The new version was used for only six samples included in this analysis.

Data Management and Statistical Analysis

A head-to-head per-sample analysis of MTB/RIF was the primary analysis for Mtb detection: the second sputum sample was examined using the MTB/RIF as the index test and the reference standard was a composite culture (LJ/MGIT) of the second sputum sample. The reference standard was positive if there was Mtb growth in at least one slope of LJ or in a MGIT culture tube, and negative if the results of both cultures - LJ and MGIT- were negative. In addition, the reference test was considered contaminated if both LJ and MGIT were contaminated. Contaminated reference standard tests or a MTB/RIF result reported as invalid were excluded from the analysis. We compared the performance of MTB/RIF with that of sputum smear microscopy. Finally, considering the LJ PM as the reference standard, we assessed the performance of MTB/RIF for the detection of rifampicin resistance and MDR-TB. Accordingly, a rifampicin resistance case was defined as rifampicin resistance detected by the LJ PM and rifampicin sensitive case was defined if the results of the LJ PM showed patterns of full drug sensitivity or drug resistance excluding rifampicin.

As a secondary analysis we conducted a head-to-head per patient assessment for Mtb detection. A subject with a positive reference standard test in at least one of the two sputum samples was considered a PTB case, and one with a negative reference standard test in both sputum samples was not considered a PTB case. Unless we refer to a ‘‘PTB case’’, all other mention of culture-positive patients or patients with tuberculosis refers to the primary analysis, thus to the second sputum sample.

Study sample size was calculated using sample size formula for estimating a proportion with a normal approximation $n= z^2.p(1-p)/e^2$ [(22)] with expectations of 98% sensitivity and 97% specificity (chosen from reports on MTB/RIF in HIV-negative patients [(21)] as at the time that our study was designed there were no studies on HIV-positive patients), 5% desired precision for a 95% confidence interval, and 5% expected attrition. No power level was specified because the primary objective was estimation and not a comparison.

All data from questionnaires and laboratory results was entered into Microsoft Access database (Microsoft, Redmond, WA, USA). Sensitivity, specificity, likelihood ratios (LLR), kappa coefficient and predictive values of the tests were calculated using 262 tables and OpenEpi v 2.3.1 [23] and the Wilson score method was used to obtain 95% confidence intervals (CI). This report was done following STARD guidelines [24].

Ethical Considerations

The study protocol was approved by the Institutional Ethics Committee at Universidad Peruana Cayetano Heredia and by the Institutional Ethics Committee at HNHU. Written informed consent was received from all participants and all data was processed anonymously. MTB/RIF results were not used for treatment management; only routine tests results were given to the treating physicians. TB cases were then referred to the National TB Program center at each site where free treatment under directly observed treatment short course (DOTS) was provided.

Results

From April 2010 to May 2011, 158 patients were screened in the two sites, of which 136 were eligible for the study and 131 were included in the analysis, as shown in figure 1. The median age of patients was 35 years (IQR 29–42) and 73% were male. The median CD4 count was 154.5 cells/mm3 (IQR 51.3–341.5). A prior TB episode was reported by 25% of patients and 32% were receiving highly active antiretroviral therapy (HAART) at enrollment. Other demographic data is listed in table 1.

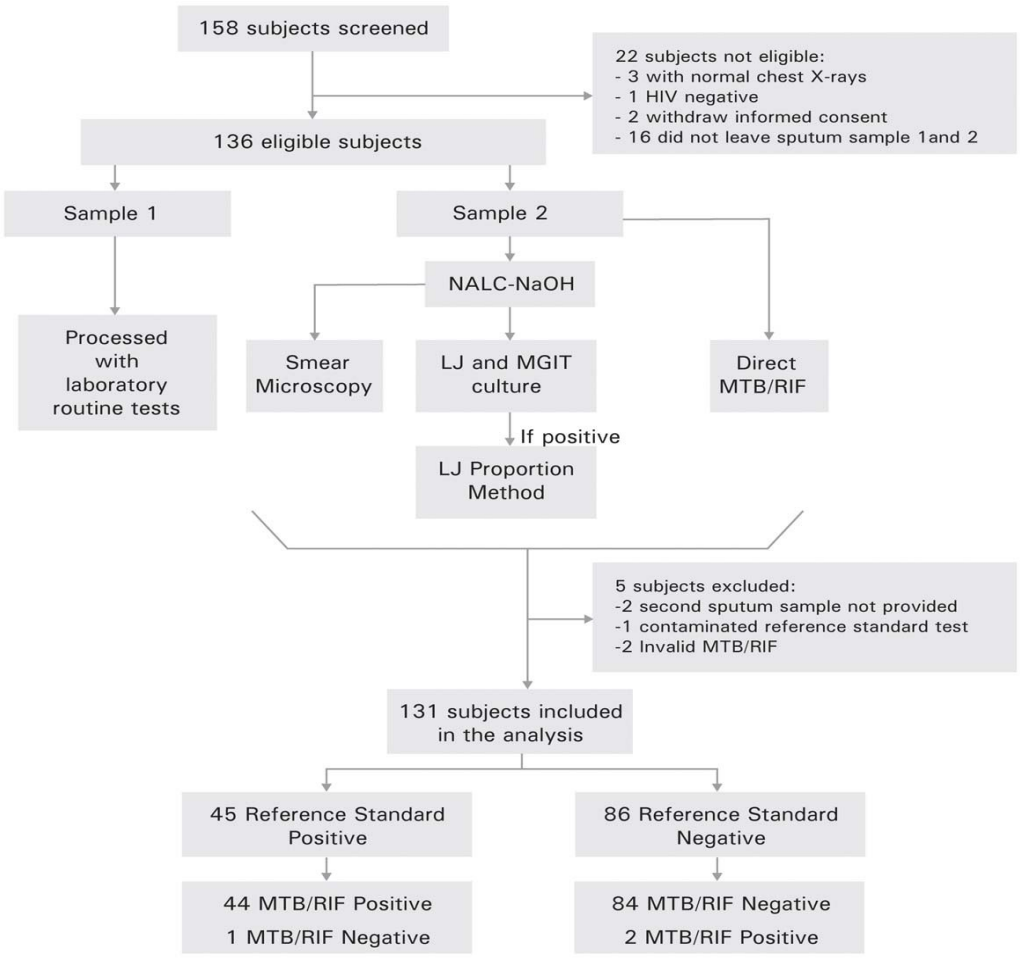
Out of the 131 patients included, 45 (34.4%) had TB and among these 14 (31.1%) were smear negative. The proportion of TB per site was 45.1% (95% CI 31.4–58.6) at HNHU and 27.5% (95% CI 18.6 –38.0) at IMTAvH.

Mtb Detection with MTB/RIF

Overall, MTB/RIF sensitivity for detection of Mtb was 97.8% (95% CI 88.4–99.6) (44/45); the specificity, 97.7% (95% CI 91.9–99.4) (84/86); the positive predictive value, 95.7% (95% CI 85.5–98.8) (44/46); and the negative predictive value, 98.8% (95% CI 93.6–99.8) (84/85). The positive likelihood ratio (+LLR) of MTB/ RIF was 42.0 (95% CI 15.8–112.1), and the negative LLR 0.0 (95% CI 0.0–0.2). The kappa coefficient value was 0.9 (95% CI 0.8–1.1).

MTB/RIF outperformed smear microscopy [97.8% (44/45) vs. 68.9% (31/45); p= 0.0002] and detected 13 out of 14 (92.2%) smear negative, culture-positive versus 31 out of 31 (100%) smearpositive, culture-positive patients. Table 2 shows the distribution of combined results according to reference standard, index test and smear microscopy for the 131 patients included in the analysis as well as three additional eligible patients with indeterminate results.

Figure 1. Study Algorithm.



LJ: Löwenstein-Jensen culture; MGIT: Mycobacteria Growth Indicator Tube; Reference standard: composite LJ & MGIT culture; MTB/RIF: Xpert®MTB/RIF; Routine tests: Smear, LJ, MGIT and LJ proportion method; NALC-NaOH: N-acetyl-L-cysteine and sodium hydroxide.

Rifampicin Resistance Detection with MTB/RIF

Six MTB/RIF cartridges with improved rifampicin resistance software (v 4.0) were used but they were all Mtb negative for both reference standard and MTB/RIF tests. Rifampicin resistance was assessed in 39 (86.7%) out of 45 patients with TB. In five cases the results of the LJ PM were not available (five LJ PM were not done, due to clerical error and one LJ PM was sensitive for all drugs, but MTB/RIF was negative for Mtb).

Table 1. Selected demographic characteristics of study patients.

	Total study patients (n=131)	Culture negative (n=86)	Culture positive (n=45)	Crude RR (95%CI)
Median age in years (IQR)	35 (29–42)	35 (30–42)	34 (29–41)	
Median CD4 count * (IQR)	154.5 (51.5–341.5)	124 (37.5–346.0)	222 (87.0–339.0)	
	n/N (%)	n/N (%)	n/N (%)	
Gender				
Male	95 (73)	61 (71)	34 (76)	1.1(0.8–1.4)
Female	36 (27	25 (29)	11 (24)	1
Prior TB episode				
Yes	33 (25)	20 (23)	13 (29)	1.1(0.8–1.5)
No	98 (75)	66 (77)	32 (71)	1
On HAART at enrollment				
Yes	42 (32)	30 (35)	12 (27)	0.9(0.7–1.1)
No	89 (68)	56 (65)	33 (73)	1
Household contact				
Yes	36 (27)	20 (23)	16 (36)	1.3(0.9–1.7)
No	95 (73)	66 (77)	29 (64)	1
Previously received isoniazid**				
Yes	15 (12)	13 (15)	2 (4)	0.4(0.1–1.4)
No	112 (85)	73 (85)	39 (87)	1

IQR: interquartile range; TB: tuberculosis; HAART: Highly active antiretroviral therapy.
*Excludes five patients with no CD4 count data.
**Excludes four patients with unknown information about isoniazid preventive treatment.

Rifampicin resistance was found in six out of 39 patients, all of these were also detected by MTB/RIF: five had MDR-TB and one was sensitive to isoniazid. Furthermore, MTB/RIF detected rifampicin resistance in three additional patients that were not detected by the reference standard. Overall, MTB/RIF sensitivity for rifampicin detection was 100%

(95% CI 61.0–100.0) (6/6); the specificity was 91.0% (95% CI 76.4–96.9) (30/33); the positive predictive value was 66.7% (95% CI 35.4–87.9) (6/9); and the negative predictive value 100% (95% CI 88.7 –100.0) (30/30). The +LLR was 11 (95% CI 5.7–21.1), and the kappa coefficient value was 0.8 (95% CI 0.5–1.1).

Outcomes of Patients with Discordant Results

In terms of Mtb detection by MTB/RIF, three (2.3%) patients had discordant results with the reference standard. A false negative MTB/RIF test was observed in a patient with a negative smear. The patient showed a positive response to treatment (defined as resolution of initial symptoms and weight gain) and was reported as cured. Two patients had false positive MTB/RIF tests, one of them was also smear-positive, responded well to treatment and was reported as cured; the other did not start treatment and died one month after study inclusion with no defined cause of death.

Finally, there were two cases with positive smears and negative results in the reference standard and MTB/RIF tests. Both of them completed TB treatment and were reported as cured.

Table 2. Combinations of smear microscopy, reference standard and MTB/RIF results within eligible patients.

Number of patients (%)*	Reference standard		Index test (MTB/RIF)	Smear microscopy	Comment
	LJ	MGIT			
30 (22.4)	+	+	+	+	Full agreement
13 (9.7)	+	+	-	-	False negative smear
1 (0.7)	+	+	-	-	False negative MTB/RIF and smear
1 (0.7)	-	-	+	+	False positive MTB/RIF and smear
2 (1.5)	-	-	+	+	False positive smear
1 (0.7)	-	-	-	-	False positive MTB/RIF
1 (0.7)	-	+	+	+	False negative LJ
82 (61.2)	-	-	-	-	Full agreement
1 (0.7)	Cont.	Cont.	-	-	Contaminated reference standard
2 (1.5)	-	-	-	-	Invalid MTB/RIF

Löwenstein-Jensen = LJ; MGIT = Mycobacteria Growth Indicator Tube; MTB/RIF = Xpert®MTB/RIF; + = positive result; - = negative result; LJ and MGIT: composite reference standard: Cont= contaminated.
*Two eligible patients were excluded because they did not provide a second sputum sample.

Three patients were MTB/RIF rifampicin resistant and LJ PM sensitive; all three

started TB treatment for sensitive cases (isoniazid, rifampicin, pyrazinamide and ethambutol for two months followed by isoniazid and rifampicin for four months). Two of them finished treatment and were reported to be cured and the other one was lost to follow-up.

Performance of MTB/RIF by Immunological Status of Patients

Performance of MTB/RIF by immunological status of the patients was evaluated for 96.2% (126/131) patients with available CD4 count at study inclusion. MTB/RIF performance was not affected by immunological status. Patients with CD4 counts below 200 cells/mm3 had a sensitivity of 100% (95%CI 83.9–100.0) (20/20) while patients with CD4 counts above 200 cells/mm3 had a sensitivity of 95.5% (95%IC 78.2–99.1) (21/22); p = 0.5. Patients with CD4 counts below 200 cells/mm3 had a specificity of 96.1% (95%CI 86.8–98.9) (49/51), while patients with CD4 counts above 200 cells/mm3 had a specificity of 100% (95%IC 89.6–100.0) (33/33); p = 0.4.

Table 3. MTB/RIF performance for *Mycobacterium tuberculosis* detection in per-patient and per-sample analysis

	MTB/RIF per-patient*	MTB/RIF per-sample[t]
Sensitivity	86.3% (95% CI 74.3–93.2) (44/51)	97.8% (95% CI 88.4–99.6) (44/45)
Specificity	97.5% (95% CI 91.3–99.3) (78/80)	97.7% (95% CI 91.9–99.4) (84/86)
Positive predictive value	95.7% (95% CI 85.5–98.8) (44/46)	95.7% (95% CI 85.5–98.8) (44/46)
Negative predictive value	91.8% (95% CI 84.0–96.0) (78/85)	98.8% (95% CI 93.6–99.8) (84/85)

MTB/RIF = Xpert®MTB/RIF
*The per-patient analysis evaluated the performance of MTB/RIF results from the second sample only against results from Löwenstein-Jensen (LJ) and Mycobacteria Growth Indicator Tube (MGIT), from both first and second sputum samples.
[t]The per-sample analysis was done on the second sputum sample and evaluated the performance of MTB/RIF against the results from LJ and MGIT from the second sputum sample.

Indeterminate Results

One patient had a contaminated reference standard test (1/134, 0.7%) with a negative MTB/RIF result. Two patients (2/134, 1.5%) had invalid results with MTB/RIF (which translates into the sample not properly processed or rt-PCR inhibited). As sufficient sample remained, MTB/RIF test was repeated in these two patients, without detection of

Mtb in concordance with the results of their reference standard.

Analysis Including Two Sputum Samples

We assessed performance of the index test for Mtb detection, done only on the second sputum sample, with a reference standard defined as any positive result in either the first or the second sputum sample to have a per-patient analysis.

A comparison of the performance of MTB/RIF for Mtb detection between per-sample and per-patient analysis is described in Table 3.

MTB/RIF sensitivity for detection of Mtb was 86.3% (95% CI 74.3–93.2) (44/51); the specificity, 97.5% (95% CI 91.3–99.3) (78/80); the positive predictive value, 95.7% (95% CI 85.5–98.8) (44/46); and the negative predictive value, 91.8% (95% CI 84.0–96.0) (78/85). The +LLR of MTB/RIF was 34.6 (95% CI 12.9–92.6) and the negative LLR was 0.1 (95% CI 0.1–0.2). The kappa coefficient value was 0.9 (95% CI 0.7–1.0).

Discussion

We report that MTB/RIF had a high specificity (97.7%) in detecting Mtb, confirming the findings of other studies [11,12,14,15]. Two patients had false positive MTB/RIF results as compared to the reference standard; however, one of them was clinically diagnosed with TB and successfully completed treatment. In low-income countries, TB diagnosis and treatment initiation is based on smear microscopy results. In our study, MTB/RIF outperformed smear microscopy for Mtb detection in almost one third of the patients. One could expect that the prompt results provided by MTB/RIF would allow a timely diagnosis and prompt initiation of TB treatment. As extensively reported in the medical literature, the benefits of rapid treatment initiation of TB in HIV co-infected patients could improve individual prognosis and reduce overall TB disease transmission [2,25]. Our results indicate much better MTB/RIF performance in Mtb detection than what has been reported by other studies on HIV patients [12–15] which may reflect some differences in study or diagnostic methodologies, or that our study population had a higher probability of TB disease, illustrated by the presence of suggestive symptoms including at least 10 day cough and chest-x ray abnormalities and the high number of patients that were not on HAART at the time of enrolment despite their compromised immunological status.

A lower sensitivity (73%) of MTB/RIF was found among patients with or without

TB symptoms in an antiretroviral therapy clinic in South Africa [15]. These patients had a lower probability of TB as opposed to our population. In a multicenter study with 40% of co-infected patients, the MTB/RIF test attained a sensitivity of 94%, similar to ours [11].

Three previous studies - two in South Africa and one in Tanzania- reported sensitivities of 70%, 84% and 88% respectively, while in our setting the sensitivity to detect TB was 98% [12–14]. These differences could also be partially explained by the fact that these studies were done on frozen stored samples. Prolonged sample storage and freeze thaw cycles may damage Mtb DNA and affect sputum viscosity, although a recent study done on frozen sputum samples described that MTB/RIF detected 64 out of 85 (75.3%) smear negative, culture-positive sputum samples, suggesting that freezing may have little impact on MTB/RIF sensitivity [26]. Nevertheless, this should be confirmed in larger studies.

Despite all the advances of HAART scale up worldwide, much of the preventable burden of TB related mortality is concentrated in populations with advanced immunosuppression and without HAART, as that of this study. Only 32% of HIV patients had initiated HAART at the time of enrolment in our study.

When we analyzed the performance of MTB/RIF compared to a reference standard including results from both sputum samples, the sensitivity was considerably reduced, yet this could be related to the fact that MTB/RIF was only done in one sample.

The performance of MTB/RIF to detect rifampicin resistance and thus its contribution for MDR-TB detection were not equally convincing. The index test did detect all the rifampicin resistant cases but also reported three false positives. Previous studies have addressed this issue [9,15,16,27] and the WHO recommends that rifampicin resistance results of MTB/RIF should be confirmed with further tests and treatment regimens should be based on the latter [28].

Our study has some limitations. WHO new guidelines recommend that TB should be suspected in any HIV-positive individual with any of the following symptoms: cough, weight loss or fever. Our study was designed before these guidelines were set, and it aimed to evaluate performance of MTB/RIF in a group of HIV-positive individuals with at least two of these symptoms, thus a more selected population. We decided to study a selected population of HIV-positive patients to narrow the risk of tuberculosis to a higher one. MTB/RIF performance could decrease among a less selected population of HIV-

positive individuals as compared to our results. Currently, MTB/RIF is still costly and targeting its use in patients with the highest risk of TB could be a strategy for resource-limited settings.

Due to resource limitations, we only evaluated MTB/RIF performance on a single sputum sample; we could not genotype the strains of three cases with false positive rifampicin resistance results. However this reflects the commonly available resources in settings with high prevalence of TB. Also, the three false positive tests were performed with MTB/RIF software v 2.1 and not with the improved software v 4.0. Nonetheless, false positive rifampicin results have been previously reported with the latest version [15]. Finally, our sample size was small for a precise assessment of MTB/RIF performance for rifampicin resistance detection. The results we report may be extrapolated to populations similar to ours but not necessarily to others with lower pre-test probability such as HIV patients without specific symptoms suggestive of TB. However these study findings suggest that in a similar setting and context an MTB/RIF negative, HIV-positive patient can be treated with high confidence.

In our study, MTB/RIF showed an excellent performance in detecting TB among patients with advanced immunosuppression and a high clinical suspicion of TB. A positive MTB/RIF result was almost 40 times more likely to occur in a subject with TB than in a subject without TB, and a negative MTB/RIF was also much more commonly seen in patients without TB.

We conclude that MTB/RIF can be an important diagnostic tool for TB disease amongst HIV-positive patients, particularly in patients with a high pre-test probability of TB. Many studies of new rapid TB diagnostic tests have been conducted in Africa where high HIV rates place a different perspective on TB programs and health systems. Further evaluation of MTB/RIF in Latin America is needed. Operational research should evaluate the yield of scaling up diagnostic algorithms of such strategies in order to evaluate the cost-effectiveness of rapid treatment initiation, improvement of individual prognosis and reduced disease transmission, within well established tuberculosis programs in TB endemic settings [29,30].

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3.3. Strengthening MDR-TB detection and treatment initiation

Otero L, Krapp F, Tomatis C, Zamudio C, Matthys F, Gotuzzo E, Van der Stuyft P, Seas C. High prevalence of primary multidrug resistant tuberculosis in persons with no known risk factors. *PLoS ONE* 2011; 6(10): e26276. doi:10.1371/journal.pone.0026276

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Paper 5

Title: High prevalence of primary multidrug-resistant tuberculosis in persons with no known risk factors

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Abstract

Introduction

In high multidrug resistant (MDR) tuberculosis (TB) prevalence areas, drug susceptibility testing (DST) at diagnosis is recommended for patients with risk factors for MDR. However, this approach might miss a substantial proportion of MDR-TB in the general population. We studied primary MDR in patients considered to be at low risk of MDRTB in Lima, Peru.

Methods

We enrolled new sputum smear-positive TB patients who did not report any MDR-TB risk factor: known exposure to a TB patient whose treatment failed or who died or who was known to have MDR-TB; immunosuppressive co-morbidities, ex prison inmates; prison and health care workers; and alcohol or drug abuse. A structured questionnaire was applied to all enrolled participants to confirm the absence of these factors and thus minimize underreporting. Sputum from all participants was cultured on Lowenstein-Jensen media and DST for first line drugs was performed using the 7H10 agar method.

Results

Of 875 participants with complete data, 23.2% (203) had risk factors for MDR-TB elicited after enrolment. Among the group with no reported risk factors who had a positive culture, we found a 6.3% (95%CI 4.4–8.3) (37/584) rate of MDRTB. In this group no epidemiological characteristics were associated with MDR-TB. Thus, in this group, multidrug resistance occurred in patients with no identifiable risk factors.

Conclusions

We found a high rate of primary MDR-TB in a general population with no identifiable risk factors for MDR-TB. This suggests that in a high endemic area targeting patients for MDR-TB based on the presence of risk factors is an insufficient intervention.

Introduction

Drug resistant (DR) tuberculosis (TB) rates are increasing globally. Access to culture and drug susceptibility tests (DST) for diagnosis remains unavailable in many settings [1,2]. Tailored case detection strategies for DR TB are required to maximize diagnostic efficiency and the rational use of financial, human and infrastructure resources. The objective of this approach is early detection of DR patients which in turn should allow early initiation of appropriate TB treatment regimens with prevention of clinical deterioration and reduction of transmission of DR strains.

Current international guidelines strongly recommend testing for DR in specific cases such as TB treatment failure, exposure to a multidrug resistant (MDR) case, return after default, relapse, in the presence of a co-morbidity, previous TB treatment with poor quality drugs or provided by a poor quality programme, and persistence of a positive smear at month two or three of a standard short course treatment [3]. In addition, DR testing is recommended in certain populations: HIV-positive patients in areas where HIV is associated with DR, prisoners, and in persons living in areas with high DR prevalence [3,4].

In Peru, DST has been centralized for many years in the national reference laboratory. National surveys from 1996 and 2006 show an increase of MDR rates from 2.4% to 5.3% among new cases, and from 15.7% to 23.6% in previously treated cases, respectively [5,6]. MDR rates are heterogeneously distributed within the country. Urban areas are most affected, with 58% of all TB cases and 82% of MDR-TB cases reported in Lima [7]. Also within Lima, a city of 9 million inhabitants, TB and MDR-TB burden is heterogeneously distributed among the districts. In most regions, DST is restricted to those with a higher MDR pre test probability [8]. This targeted testing has shown good results: in two districts of Lima, 34% of patients with at least one risk factor for MDR had indeed MDR [9]. A good selection of patients to be tested based on their pre test probability is a component of an efficient diagnostic process. In order to contribute with evidence for enhanced MDR case detection strategies, we aimed to evaluate the yield of testing low risk patients living in a high MDR burden area.

Materials and Methods

Study setting

The study was conducted in a semi-urban district in Lima with a population of over

one million inhabitants [10]. The district has a TB incidence of 213 per 100,000 inhabitants [11] and a MDR prevalence of 7% among all TB cases in the area [12]. Social security and private health care facilities also operate in the district. However, most TB cases are managed in the public health services [13]. The HIV prevalence in TB patients in this setting is similar to the national prevalence, which in 2008 was 2.6% [14].

Patient recruitment was conducted in all 34 public health care facilities (one hospital and 33 first level health care facilities) of the district. The NTP has designated offices in every facility. Following national guidelines, TB suspects with at least one sputum sample positive for acid-fast bacilli are started immediately on TB treatment. Sputum culture and DST for first line drugs are routinely requested for any retreatment category (failure, return after default, relapse); persons reporting exposure to a MDR-TB case or to a TB case that failed treatment or that died during treatment; patients with immunosuppressive co-morbidities such as HIV and diabetes; persons working or admitted in a prison; health care workers, and persons with a recent and prolonged admission to a hospital [8]. Sputum samples from new TB patients who do not report one of these high risk factors for MDR are not cultured routinely. These patients start a regimen for drug susceptible TB which consists of two months of isoniazid, rifampicin, ethambutol and pyrazinamide taken six days per week followed by four months of isoniazid and rifampicin taken two days per week. During follow-up, patients are evaluated monthly for smear conversion; if smears remain positive at month two or three, a sputum sample is sent for culture and DST. A MDR consultation committee decides further management, usually starting a standardized second line regimen until full DST results are available for individualized regimens.

Patient recruitment

From April 2008 to March 2010, adults diagnosed in the 34 health facilities with a first episode of smear positive TB and no reported risk factors for MDR-TB were eligible and invited to participate in the study. Trained field workers covered from Monday to Saturday from 8 am until 2 pm the facilities with large numbers of TB patients and twice or thrice a week those with fewer patients. They first screened eligible patients. We considered risk factors for MDR-TB all the criteria that the national guidelines use to request a culture and a DST among new smear positive cases, as well as alcohol and drug abuse. Patients reporting at least one of these factors were not enrolled. To correct for

underreporting during screening we subsequently looked into more detail for these risk factors, and previous TB treatment, with a structured questionnaire applied to every participant enrolled. If a risk factor was still elicited in enrolled participants, all study procedures would be continued but these participants would be excluded in the analysis. Demographic data, exposure to TB, and clinical data such as use of previous prophylaxis was recorded for participants. Field workers instructed the patient on submitting a sputum sample. Patients submitting samples after taking more than three doses of anti tuberculosis treatment were also excluded.

If the patient had been recently tested for HIV, the result was retrieved from the clinical files. The NTP recommends and offers HIV testing to all TB cases. When the patient had not been tested recently, the field workers offered the patient the option to be screened. HIV positive patients were referred to the national HIV programme for management. HAART is provided free of charge to patients. Pre and post test counselling are done by qualified staff from the Ministry of Health.

Sample collection and processing

If the patient could not produce a sputum sample on the spot, a morning sample was collected the next day. Sputa were transported within six hours to the microbiology laboratory at the Instituto de Medicina Tropical Alexander von Humboldt. Samples were kept at room temperature and processed on the day of arrival. If a sample arrived in the afternoon or on a Saturday, it was kept at 4°C until the next day or until Monday. A Ziehl-Neelsen smear was done, and the sputum was cultured in two slopes of Löwenstein-Jensen media. Positive cultures were tested for drug susceptibility using the 7H10 agar method and growth with the following drug concentrations of antibiotics defined resistance: 0.2 µg/ml isoniazid, 1.0 µg/ml rifampicin, 6 µg/ml ethambutol and 2 µg/ml streptomycin.

A sample of 74 mycobacterial isolates was sent for external quality control of DST to the Institute of Tropical Medicine in Antwerp, Belgium, a WHO/IUATLD supranational reference laboratory.

Statistical analysis

Data were entered in Microsoft Access database (Microsoft, Redmond, WA, USA), and analyzed with EpiInfo version 3.5.1 (CDC, Atlanta, GA, USA). A TB patient was the unit of analysis and the DR pattern the outcome variable. The definition of a MDR case

was resistance to isoniazid and to rifampicin on at least one Löwenstein-Jensen slope. ‘‘MDR-plus’’ was defined as MDR and resistance to another drug, ‘‘mono resistance’’ was defined as resistance to a single drug out of the four tested, ‘‘poly resistance’’ was defined as resistance to at least two drugs not involving the combination of isoniazid and rifampicin. The MDR rate was the proportion of MDR-TB cases among the patients that had a DST result.

Proportions in subgroups were compared using Pearson’s Chi square test; odds ratios (OR) were calculated with 95% confidence intervals (95% CI).

Ethical considerations

This study was approved by the Institutional Ethics Committee at Universidad Peruana Cayetano Heredia and by the Regional Health Direction from the Ministry of Health. Written informed consent was obtained from all participants. All data were processed anonymously. A separate informed consent was used if the patient was to be tested for HIV.

Results

We enrolled 909 patients with a first episode of smear positive pulmonary TB that did not report a high risk factor for MDR-TB during screening. After thorough questioning, 203 did report at least one high risk factor for MDR-TB not detected initially and 34 did not give full information. Out of those enrolled but at known risk for MDR, 198/203 (97.5%) had a culture result available of which 177/198 (89.4%) were positive. MDR rate among this group was 13.6% (24/177). None of the participants with incomplete information on risk factors had MDR-TB. From the 672 not reporting a risk factor 13.1% (88) were excluded as they did not have a positive culture. Finally, among participants not reporting a risk factor that had a positive culture, 6.3% (95% confidence intervals (95%CI) 4.4–8.3) (37/584) were MDR. Enrolment process and MDR rates are shown in figure 1.

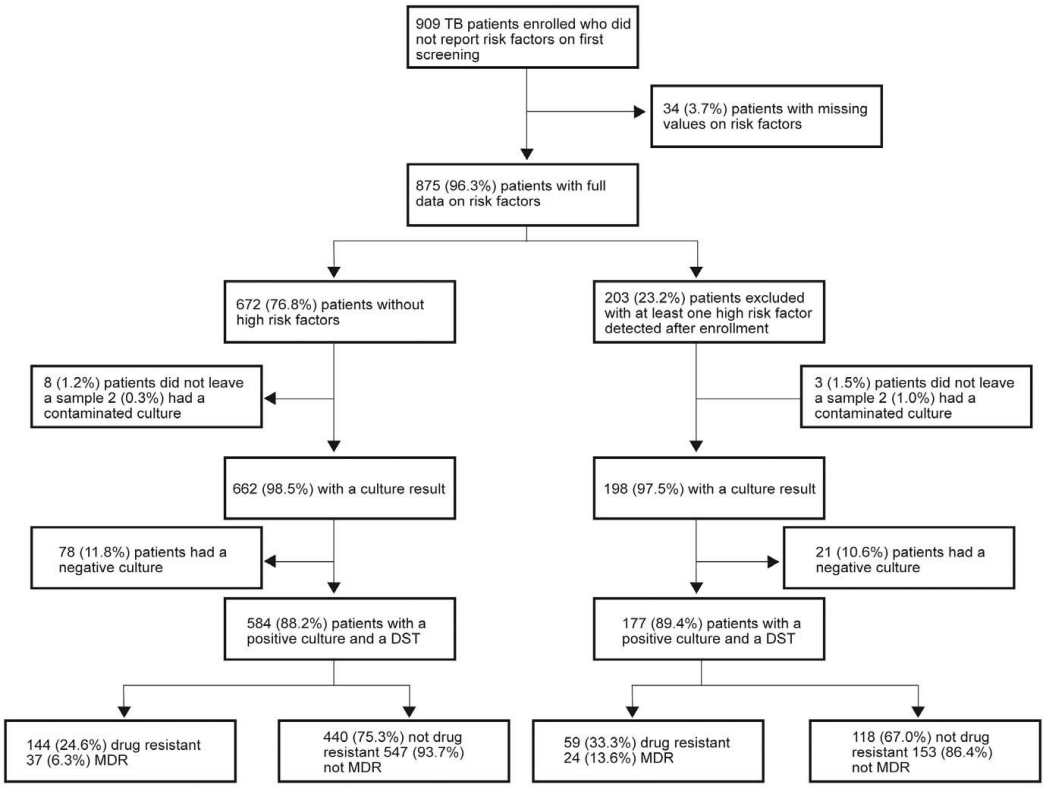
Of the isolates sent for DST external quality control, it was possible to culture 59/74 (79.7%) again. Concordance for isoniazid and rifampicin resistance profiles was 100% and 93.1% for ethambutol.

DST patterns for the low risk population are shown in figure 2.

Overall, 117/584 (20.0%, 95%CI 16.8–23.3) were resistant to isoniazid and among the non MDR patients, 80/547 (14.6%, 95%CI 11.7–17.6) were resistant to isoniazid, either mono or poly resistant.

Among the 584 patients at low risk of MDR, 391 were HIV negative and 193 had an unknown status. The MDR-TB rates in both groups were similar: 6.7% (95%CI 4.2–9.1) (26/391) and 5.7% (95%CI 2.4–9.0) (11/193), respectively. Table 1 shows the patient's characteristics associated with MDR-TB without known risk factors compared to those with either pan susceptible or with DR patterns other than MDR. We found no significant factors associated to MDR. Out of fourteen patients that reported previous use of isoniazid prophylaxis, none were MDR, six were resistant to isoniazid (five were mono resistant and one was poly resistant), and the other eight were pan susceptible.

Figure 1. Patient enrolment and drug resistance results.

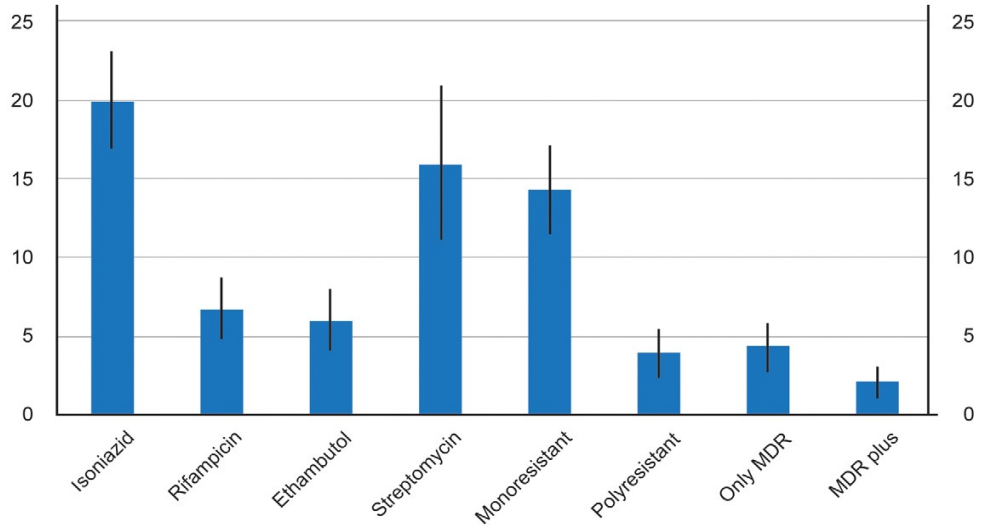


Legend: DST = Drug susceptibility test; Drug resistant = any drug resistance, including MDR; MDR = multidrug resistant.

Discussion

High MDR rates were found among new smear positive pulmonary TB patients without known risk factors for MDR. This finding suggests that patients are at risk of infection with a MDR strain by living in a high incidence area where ongoing transmission takes place. The high burden of MDR shown in our data from an urban population may be masked in national surveillance or in national surveys of drug resistance based on a representative sample of TB patients in the country, when areas of low MDR rates predominate. These results should prompt further investigations in other districts of Lima and regions of Peru. The course of action derived from this study should be universal access to DST for populations living in districts with high incidence of MDR-TB.

Figure 2. DST patterns in patients with no reported risk factors for MDR-TB: rates and confidence intervals.



Legend: Mono resistant: resistance to a single drug out of the four tested; Poly resistant: resistance to at least two drugs not involving the combination of isoniazid and rifampicin; Only MDR: resistance to isoniazid and rifampicin only; MDR plus: resistance to isoniazid, rifampicin and another drug.

The study has some limitations. Only patients attending public sector health care facilities were included. However, in Peru, most cases of TB are treated within this sector [13]. Also, the selection of patients without risk factors relied on patient's report, which could have masked some risk factors. In particular, patients may not have reported previous TB treatment which might overestimate the rate of MDR among new cases. To limit under reporting, once enrolled, exhaustive probing for each factor was done. Finally, we might

have lacked power to detect association with weak risk factors such as isoniazid prophylaxis. Nevertheless we have a representative population from a highly populated district as all public health care facilities were included. The negative culture results in a group (11%) of enrolled patients might have been caused by sterilization after taking one or two doses of rifampicin, or by a failure in the transport or decontamination procedures set for the study [15].

Table 1. Characteristics and MDR status of patients without known risk factors.

Exposure variable	N	MDR	Non MDR	Crude OR (95% CI)
		N (%)	N (%)	
Age median (IQR)		26.02 (23.16–32.04)	27.52 (22.58–36.56)	
<25	226	16 (7.1)	210 (92.9)	1
25-34	197	13 (6.6)	184 (93.4)	1.08 (0.51-2.30)
>35	159	8 (5.0)	151 (95.0)	1.44 (0.60-3.45)
Sex				
Male	308	19 (6.2)	289 (93.8)	
Female	268	18 (6.7)	250 (93.3)	1.09 (0.56–2.13)
TB contact *				
No	337	20 (5.9)	317 (94.1)	
Yes	247	16 (6.5)	231 (93.5)	1.09 (0.56–2.16)
Previous prophylaxis**				
No	535	34 (6.4)	501 (93.6)	
Yes	14	0 (0)	14 (100.0	0.5 (0.003–0.89)**
Tobacco use				
No	486	30 (6.2)	456 (93.8)	
Yes	79	7 (8.9)	72 (91.1)	1.48 (0.63–3.49)
Residency within the district				
Upper area	312	16 (5.1)	297 (94.9)	
Lower area	272	21 (7.7)	251 (92.3)	1.55 (0.79–3.04)

*Patient states having been in contact with a person with active TB who is alive and whose episode of TB was not report to be MDR-TB.
**To calculate this odds ratio (OR), 0.5 was added to each value, and the standard formulae for OR, confidence intervals and p-value were applied.

The risk factors associated with primary MDR-TB from other studies cannot be compared to our results as patients with known risk factors were excluded. We did not find any additional variable associated with primary MDR-TB. TB treatment failure is the strongest factor associated to MDR [9,16–19] followed by exposure to an MDR-TB case or to a case failing treatment. Female sex has been associated with MDR-TB in some settings

[18,19] as well as lung cavities [20,21].

Enrolled patients with risk factors had a high rate of primary MDR (13.6%). This rate is most probably underestimated as this study only enrolled patients with high risk factors that were not reported during initial screening. The cost effectiveness of testing all TB cases in high MDR burden areas and only high risk groups in low risk areas for MDR-TB should be evaluated. The feasibility of testing large numbers of patients, the tests to be used, and the impact on the efficiency of local, regional and reference laboratories should also be considered. Introduction of expensive rapid methods should probably be restricted to settings where their positive predictive value will be high. Except when extremely specific, they will yield a high proportion of false positives in areas with low MDR prevalence and may not be a cost effective as a screening tool.

The high proportion of primary MDR found among patients without risk factors attending primary health care facilities in a populated district in Lima suggests transmission of drug resistant strains among the general population. This indicates a need for scaling up strategies to reduce transmission of MDR-TB: early detection, proper management of MDR-TB cases, infection control measures, and addressing the high defaulter rates from both sensitive and resistant TB. This study suggests that in a high endemic area targeting patients for DST based on the presence of risk factors is clearly an insufficient intervention for early case detection of MDR-TB.

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Paper 6

Title: Time to initiation of multidrug-resistant tuberculosis treatment and its relation with outcome in a high incidence district in Lima, Peru

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Abstract

Objective

To determine the time from diagnosis to start of multidrug resistant tuberculosis (MDR-TB) treatment in Lima, Peru.

Methods

We studied new smear-positive TB adults that were started on MDR-TB treatment or that were switched to it between June 2008 and December 2011.

Results

Time from the first positive smear to MDR-TB treatment was >30 days in 35% (13/37) of patients. Among the 27% (24/88) of patients that switched to MDR-TB treatment, time from the last dose of a drug-susceptible regimen was >30 days.

Conclusion

Start of and switching to MDR-TB treatment is still delayed.

Introduction

Prompt start of treatment for multidrug-resistant tuberculosis (MDR-TB) is essential to achieve cure and to reduce transmission. The diagnostic delay attributable to the long turnaround time of conventional culture and drug susceptibility testing (DST) can now be overcome with molecular tests that diagnose MDR-TB in 90 min (WHO 2013). However, to entail clinical and public health impact, diagnosis must be followed by rapid initiation of the appropriate treatment, adherence to it and completion (Davis et al. 2012; Pai et al. 2012).

Multidrug-resistant tuberculosis treatment is complex because of its length and rate of adverse events. Cure rates are lower than those of drug-sensitive TB (Orenstein et al.2009). MDR-TB treatment can be individualised to a patient's DST or standardised to DST patterns based on national surveys. In Peru, the progressive strengthening of MDR-TB management by the National TB programme (NTP) and Reference Laboratory (NRL) included the scaling up of faster DST and the decentralisation of MDR-TB care (Yagui et al. 2006; Shin et al.2008). We determined the time span between TB diagnosis and initiation of MDR-TB treatment in Lima, Peru, to identify possible delays.

Methods

Study setting

The study district's annual incidence for all TB forms is 95 per 100 000 inhabitants, and 8% of new patients have MDR-TB (Ministerio de Salud 2010). The Ministry of Health provides free TB care in 34 facilities under directly observed therapy. The NTP establishes predetermined criteria to identify MDR-TB suspects: previous TB treatment, immunosuppression, exposure to prisons or health facilities, contact with an MDR-TB case or of a TB case that failed treatment or died, and persistent positive smears during treatment. In addition, the health facility staff may consider that a patient is at risk of MDR-TB in the absence of one of the NTP pre-established criteria.

At the study district, apart from the Löwenstein-Jensen proportion method, a rapid nitrate-reductase colorimetric assay is performed since 2007. It is a low-cost DST method that uses conventional materials and when carried out on a smear-positive sputum sample, results are available in 21–28 days.

In parallel, the chest physician participates in a clinical evaluation expert committee that meets periodically for evaluation of the patient's files to design the individualised regimen. If neither the patient nor a close contact has a DST, a standardised regimen is started. This standardised regimen is constructed on the basis of national drug susceptibility surveys.

Study design

We retrospectively studied new smear-positive pulmonary TB adults diagnosed in the district between June 2008 and December 2011 that received a MDR-TB treatment: those who were started on it were defined as "starters" and those who were switched to it after starting a standard regimen for drug susceptible were defined as "switchers." We reviewed clinical files and patients' records for the treatment start and end dates as well as the regimens used.

Statistical analysis

For "starters," we calculated the time from the first smear positive result to the first MDR-TB treatment dose. For "switchers," we calculated the time from the first smear positive result to the first dose of the drug-susceptible regimen and the time elapsed between the last dose of it and the first dose of MDR-TB treatment. Times to initiate and switch to MDR-TB treatment were tested against treatment outcomes which we classified as favourable (cure or treatment completion) and adverse (death, default, failure or transfer out).

Ethical considerations

The Institutional Review Board at Universidad Peruana Cayetano Heredia and at Antwerp University approved this study.

Results

During the study period, 127 patients were treated for MDR-TB: 37 (29%) "starters" and 90 (71%) "switchers." At least one NTP pre-established criterion to request a DST was registered in 30 (81%) "starters," but also in 31 (34%) 'switchers'. The date when the treatment was changed was not available for two "switchers," and they were excluded from the analysis. Median times to initiation and switching of treatment are

shown in Table 1. MDR treatment was initiated after more than 30 days in 35% (13/37) of 'starters'. Among "switchers," the time between treatment regimes was 1 day in 18% (16/88) of patients, 1–7 days in 26% (23/88) of patients, 1–4 weeks in 30% (26/88) and over 1 month in 26% (23/88) of patients. No treatment outcome was available for 5.5% (7/127): four were still on treatment and three had missing information. Among the remaining 120 patients, a favourable outcome was present in 26 (77%) "starters" and in 55 (64%) 'switchers'; eight (24%) of the "starters" and 31 (36%) of the "switchers" had an adverse outcome (RR 1.2 (95% CI 0.9–1.5). Overall, 23 (19%) patients defaulted. Median time to MDR-TB treatment initiation among "starters" was similar in those with an adverse outcome, 25 [interquartile range (IQR), 18–30] days and, in those with a favourable outcome, 26 (IQR, 18–41) days (P = 0.6). Among "switchers," the time to switch was 11.5 (IQR, 2–35) days in those with a favourable outcome and 22 (IQR, 2–48) days in those with an adverse outcome (P = 0.1).

Table 1 Time to initiation and switch of treatment regimens

	Time in days				
	N	Median	25^{th}-75^{th} percentile	Min	Max
Patients starting on MDR-TB treatment					
Between first positive smear and MDR-TB treatment	37	25	16-41	3	81
Patients starting on DS-TB treatment					
Between first positive smear and DS-TB treatment	90	4	2-7	-5	28
On DS-TB treatment	88	105	60-157	14	238
Between last dose of DS-TB and first dose of MDR- TB treatment	88	11.5	2.5-35.5	1	124

MDR-TB, multidrug-resistant tuberculosis; DS-TB, drug-susceptible tuberculosis

Discussion

In our study, initiation of MDR-TB treatment took more than one month in 35% of the "starters." Likewise, among "switchers," the period in between regimens was generally long. We did not have sufficient power to demonstrate an impact of time to treatment initiation on outcomes.

The reduction in diagnostic delay achieved by tools, such as the nitrate-reductase colorimetric assay and even more so with molecular assays, has apparently not been echoed by a sufficient reduction in treatment delay. For instance, in two recent South

African studies conducted under routine conditions, genotype MTBDRplus reduced the laboratory processing time but not the time to notification of the results; median time between sputum collection and MDR-TB treatment was 55 and 62 days (Hanrahan et al. 2012; Jacobson et al. 2013). Also in South Africa, 85% of patients from a paediatric cohort had a median time of 100 days between MDR-TB diagnosis with Xpert®MTB/RIF and treatment initiation (Theron et al. 2014). Compared to the studies above that used molecular methods, we found a much shorter but still considerable time to MDR-TB treatment initiation. The reduction in the delay may be a result of the NTP and NRL efforts in recent years to improve MDR-TB management such as decentralising laboratory infrastructure and decreasing bureaucracy between first line health services and expert committees that design individualised treatment regimens. Despite these efforts, treatment delay has been reduced but not to a negligible length. The 30-day delay in 35% of "starters" is partly due to the time required for the periodically meeting expert committees to decide on the regimens for the treatment becoming available at the health facility. Also, patients have to undergo baseline laboratory tests and medical consultations before MDR-TB treatment is started. The NTP has made these evaluations free of cost for patients, but scheduling of appointments can still take a few days.

Reductions of the delay of MDR-TB diagnostic obtained with rapid DST will translate into swift treatment initiation if the steps that follow MDR status determination ascertainment are concurrently addressed. The evaluation of the patient before MDR-TB treatment initiation, the decision on and prescription of the regimen, the availability of the drugs, to name but a few, depend on multiple persons and components of a health system. Setting up efficient organisational flows managed by trained staff equipped with the resources required could enhance the impact of the introduction of new diagnostic tools and improved MDR-TB treatment regimens (Clouse et al. 2012; Pai et al. 2012).

In recent studies, implementation of molecular tests did not reduce TB morbidity and mortality (Hanrahan et al. 2012; Theron et al. 2014). A delayed start or switch to MDR-TB treatment could be playing a role in individual treatment outcomes. In addition, provider delay weakens the messages to patients on the importance of adherence to achieve bacteriological clearance and to avoid acquisition or amplification of resistance.

When this study was concluded, the NTP had issued new guidelines recommending universal DST with rapid, including molecular tests. This constitutes an

opportunity to further reduce delays in all patients and should reinforce MDR-TB management. We found that in an urban district, where faster diagnostic tests for MDR-TB were already implemented, start of and switching to MDR-TB treatment were still delayed. This study emphasises that implementation of improved technologies needs simultaneous implementation of strategies to speed up treatment initiation.

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4. General discussion, conclusions and recommendations

4.1. Main findings

We found gaps and inefficiencies in the case detection process of TB and MDR-TB patients in Lima, Peru. Our results show that case detection could be optimized by bridging the gap between policy and practice of existing guidelines, by policy changes and by improving use of program monitoring data.

Our findings illustrate that the second component of the DOTS strategy (see box in chapter 1.2) -case detection through quality assured smear microscopy- is implemented suboptimally and its consequences go unnoticed in routine monitoring. We demonstrated that the Peruvian National TB Programme definition of TB suspects performs well in detecting smear-positive patients. However, it was not applied consistently. Half of the persons tested were not TB suspects and had a low smear-positivity yield compared to TB suspects (3.2% vs. 12.4%); this rendered the screening process inefficient. The technical quality of smear microscopy was also suboptimal. We implemented a new sampling procedure to increase efficiency of the quality control system of smear microscopy. It efficiently detected laboratories with quality control problems but improvements were not seen in the second year because of lack of feedback from supervisor to laboratory technicians.

As for case detection in groups at higher risk of TB, the second component of the Stop TB strategy, we found that TB incidence among household contacts of pulmonary TB patients was ten times higher than that of the general population and that extending the surveillance period of household contacts from diagnosis of the index case to up to two years would increase the number of TB cases detected. Incident TB was significantly more frequent among male household contacts, siblings and partners to the index case. Household contacts of index cases with highest bacillary load in the sputum and a time between start of symptoms and TB treatment initiation of over 30 days had significantly more incident TB. We found that case detection among HIV-positive TB suspects could be improved by using Xpert®MTB RIF test. The molecular test had a high sensitivity (97.8%) and specificity (97.7%) to detect TB among HIV-infected patients with a high probability of TB and it significantly outperformed smear microscopy (97.8% vs. 68.9%).

MDR-TB case detection and treatment initiation was suboptimal. The proportion of

MDR-TB among TB cases with no known risk factors living in the study district, with high prevalence of MDR, was higher than the national average. The suspect definition in use at the time of the study comprising only known risk factors for MDR-TB – as per guidelines at the time of the study– missed many cases. Furthermore TB diagnosis did not translate into immediate appropriate treatment: in a third of patients, MDR-TB treatment was delayed over 30 days after the first positive smear was obtained at the health facility.

4.2 Strengths and limitations of the thesis

The surge in recent years on research in new diagnostic tests for TB has not been complemented with research on the case detection process. If the case detection process is suboptimal, even a well performing diagnostic test will not lead to early diagnosis of most patients. We determined gaps and potential solutions that National TB Programmes can use to improve the case detection process in Lima, Peru and similar settings. We obtained a comprehensive picture of the case detection process in first line health facilities in a large district in eastern Lima and studied specific components of the Stop TB Strategy. This was facilitated by studies under research-controlled conditions and under routine conditions, and by using different study designs: cross sectional, prospective and retrospective longitudinal.

Five of the six studies of this thesis were conducted in the same district, San Juan de Lurigancho. The problem of TB suspects and cases that never reached health services was outside the scope of the study. Few studies have addressed this issue because of the inherent complexity of doing so. However, in a setting where access and acceptability of health services is low, many TB suspects may not be reaching the first step of case detection. Geographical access does not seem to be a barrier in San Juan de Lurigancho where there are 34 health facilities distributed throughout the district area. However, unstable job conditions and informal economic activity may reduce timely access of suspects to health facilities. Suspects do not have to pay for TB and MDR-TB diagnostic tests and treatment, but the opportunity cost of attending the facility while missing their job may be high. Opening hours at health facilities are usually short and waiting times are usually long. Although cultural issues are not a major problem in urban Peru, except perhaps for recent rural migrants, perceived quality of care is low among people seeking health care at the public health system, including TB services. Counselling and support for the emotional and psychological burden of a potential TB diagnosis as well as health

education is very weak. Suspects may be lost in the TB detection process because they may be told to provide sputum samples and return for the results, but they may not be informed on the reasons to do so and on the benefits of timely treatment if TB is confirmed. San Juan de Lurigancho is the district in Peru with the largest population but it may not be representative of others areas. The TB case-load of San Juan de Lurigancho is larger than many other districts and therefore gaps observed in the case detection process may reflect to some extent this higher burden.

Most of our findings lead to direct and concrete recommendations. However, we did not further explore some of the unexpected gaps and inefficiencies found. In the first study, we did not query why study participants that did not meet the TB suspect definition submitted a sputum sample, we did not register the quality of sputum samples and its association to smear positivity and we did not estimate the costs of testing people with less than two weeks of cough. In the fifth study, we did not determine the different components contributing to the delay in treatment initiation of patients suspected of MDR-TB. Therefore, some explanations for our findings are hypothetical and should be further investigated.

4.3 Implications for control practices

4.3.1 Augment efficiency of diagnosis of TB suspects with smear microscopy

The limited sensitivity of smear microscopy to diagnose TB can be balanced by using laboratory tools that improve its performance and by an effective quality control system. However, in a global survey, National TB Programme managers stated that a frequent problem they faced was difficulty in convincing laboratory staff regarding the benefits of tools and strategies to improve smear microscopy. Evidence on straightforward strategies to augment efficiency of TB diagnosis such as increasing the pre test probability of persons tested and making more efficient quality control systems can support decision-makers for policy changes.

Laboratories are overloaded with unnecessary samples from persons who are not at risk of TB and technical quality control of smear microscopy is limited by lack of feedback and supervision. Persons that were not TB suspects were likely tested in order to achieve the monthly screening targets set by National TB Programme authorities (5% of all

adult consultations) in 1990. These targets have not been changed despite the halving of TB incidence. In 1990, the proportion of TB cases among suspects tested was 11% (24,023/210,905), while in 1999, it went down to 2.3% (24,611/1'085,749) (1). Furthermore, not reaching screening targets can result in retaliation against the staff instead rather than investigation of the factors contributing to not meeting the goals (2). This TB Programme performance target is hindering the monitoring of activities. Managers may think the program is doing well by screening a large number of persons. However, it is done in a very inefficient manner. We recommend revising the target of TB suspects to be screened per month to match the current epidemiology. Front line staff should be instructed to only test TB suspects, with exceptions made on an individual basis. The definition of TB suspects as persons coughing for more than two weeks applies only to HIV negative persons. HIV positive persons with any duration of cough or the presence of fever, night sweats or weight loss should be tested for TB (9).

After implementing the modified EQA no improvements were seen because the supervisor provided poor and scarce feedback to the peripheral laboratory technicians on their reading as has been observed in other settings (3). Supervision –systematic review by a laboratory expert- is signaled as the most important component of a strong laboratory network (4) but it is labour-intensive and complex. Supervisors need to be well trained and motivated to conduct their activities. Frequently, they do not know how to supervise, as there are no standard procedures for its implementation. Also, supervision is limited by a culture of avoiding bad results instead of taking corrective actions. Mistakes or underperformance cause fear of retaliation rather than a desire to understand the source of the problem and make the appropriate intervention to solve it. Finally, there is no accountability for work of laboratory staff. This has serious implications on TB case detection, because systematic and non-systematic errors on smear microscopy go unnoticed. Our findings illustrate how EQA is dependent on good performance of all involved and the central role of feedback on results (3–5).

Selection of TB suspects and case detection with quality assured smear microscopy are not automated processes and depend on the diligence and motivation of staff. This results in suboptimal use of the most used tool for TB diagnosis. We recommend optimizing the selection of suspects and addressing the gaps in quality control. Not doing so could lower the expected effect of implementing better performing tests.

4.3.2 Enhance case detection in specific groups at increased risk of TB

While the largest burden of TB patients will be detected through passive case detection as they become symptomatic, certain groups have a sufficiently higher risk of TB to justify enhanced or active case finding. Household contacts of recent TB cases are the group in which the highest yield of TB cases is found with active case finding (6,7). Likewise, HIV patients are at higher risk of complications caused by TB, especially death. Intensified case finding is recommended among them as well as the use of tests with higher sensitivity than traditional methods that underperform in advanced immunosuppression (8,9).

Household contact investigation poses a particular challenge to TB programs. TB is very frequent among household contacts but in medium and high incidence areas they are a small proportion of all TB cases. As household contacts are most often asymptomatic, their motivation to attend screenings is low and staff has to actively look for them. This places a large workload on teams in TB clinics, which most often conduct partial investigations. The Peruvian National TB Programme recommends three medical evaluations for each contact throughout the six-month treatment regimen of a drug susceptible index case. However, as most recent TB infection is activated within two years after exposure, as supported by our findings, a two-year surveillance for TB among household contacts is justified. Targeting investigation to only some contacts or only some households –on the basis of the differential risk we found - may reduce the amount of time required to do it, but could also add more complexities to the planning as they would have to be selected beforehand. Also, staff, TB cases and contacts may not find acceptable that not all households or household contacts are screened.

On the basis of our results, we recommend extending household contact investigation up to two years but not necessarily with face-to-face visits and certainly not by imposing major additional workloads on TB staff, unless teams increase in number. Mobile technology could decrease workload of consultations and home visits while maintaining awareness of TB risk among contacts and motivation to seek care if symptomatic. Several successful health interventions in Peru have exploited the 91.4% penetration of mobile phones countrywide (10).

The unusual epidemiological context in Peru of low HIV prevalence, medium TB

incidence and high MDR-TB prevalence has resulted in centralized care of HIV patients in referral hospitals where the use of more advanced diagnostic technologies for TB is feasible. Xpert®MTB/RIF is particularly useful among groups in whom the speed of diagnosis is important because of poor prognosis if treatment is delayed. Such is the case of HIV co infected patients and drug resistant TB patients. HIV positive patients also benefit from being tested with Xpert®MTB/RIF because smear-negative TB is frequent in this population and Xpert®MTB/RIF outperforms smear microscopy. Furthermore, hospital admissions among HIV patients with undiagnosed TB or MDR-TB pose a threat for nosocomial outbreaks. Expanding the use of this rapid method could accelerate TB treatment initiation among HIV positive patients and reduce the risk of nosocomial transmission. Also, by increasing accuracy in the ruling out of active TB among HIV patients, the prescription of isoniazid prophylaxis could increase (11). Isoniazid prophylaxis is recommended to all HIV patients that do not have active TB, but ruling active TB out is an obstacle to its prescription (12,13).

However, implementation of Xpert®MTB/RIF in Peru needs careful consideration. The Peruvian National TB Laboratory Network executes four rapid DST (MODS, Genotype®MDRTBplus and Griess) in different regions in the country. In addition, all samples tested with these rapid DST are confirmed with the Löwenstein-Jensen proportion method, or MGIT, that also provides a full drug susceptibility pattern of second line drugs. Also, rifampicin mono resistance is frequently used as a proxy for MDR-TB. However, a non-negligible proportion of rifampicin mono resistant TB occurs in San Juan de Lurigancho (14). Xpert®MTB RIF does not test for isoniazid. Therefore mono resistance to rifampicin in patients may be misclassified as MDR-TB where confirmatory testing is not available.

We recommend implementation of Xpert®MTB/RIF only if it is a replacement for an underperforming DST and not an addition to the six methods currently used. Implementing a new test within a program is a complex task that goes beyond performance because each additional test, requires a procurement system and a quality control system (4,15). The already implemented Genotype®MDRTBplus tests for isoniazid and rifampicin resistance and its performance is similar to that of Xpert®MTB/RIF but it is only used among smear-positive TB patients (16,17).

4.3.3 Strengthening MDR-TB detection and treatment initiation

Halting transmission of MDR-TB strains through early diagnosis and treatment initiation is essential given the poor treatment outcomes of MDR-TB, adverse events of second line drugs and high costs of treatment (18,19). Costs of DST are still prohibitive in many settings where laboratory capacity is limited. Where universalization of DST cannot be afforded, testing groups with a higher pre-test probability of MDR-TB is a feasible strategy. Risk groups should be studied in context and periodic DST surveys can be used to monitor trends of drug resistance among general population. The proportion of MDR-TB in a certain group will guide this selection but the size of the group should also be considered. For example, the former Peruvian definition of MDR-TB suspects had a low sensitivity but very high specificity. MDR-TB was very frequent in the groups defined as suspects but those groups were small in size (e.g. prison workers, health care workers, MDR-TB contact).

In November 2013 and after the publication of our study, the Peruvian National TB Programme expanded its DST policy to all TB patients. This policy will contribute to halting transmission by increasing the number and speed of MDR-TB cases diagnosed. Universal DST testing allows accurate classification, early MDR-TB treatment, appropriate management of contacts and protection of both staff and community by halting transmission (20–22). In addition, universal DST contributes to TB control by providing surveillance data to monitor drug resistance trends.

Early diagnosis of all MDR-TB cases will only contribute to preventing transmission if treatment initiation of MDR-TB is swift. Although we found a shorter MDR-TB treatment delay in San Juan de Lurigancho as compared to other settings, the Peruvian National TB Programme needs to reduce it further both among those that are started on MDR-TB regimens as well as among those who are switched to MDR-TB treatment after stopping a drug sensitive regimen. A delay in treatment initiation by the TB treatment team may contradict the message given to patients regarding the importance of treatment initiation and strict adherence to achieve bacteriological clearance, to halt transmission and to avoid acquisition of resistance. It is likely that in our setting, the complexity of individualising MDR-TB treatment to DST is the main contributor to treatment delay. If the logistical complexity of starting individualised MDR-TB treatment is not minimized, its therapeutic advantages over standardized MDR-TB treatment may be

reduced. A meta-analysis of 34 studies on MDR-TB treatment outcomes did not find an association with therapeutic and programmatic factors such as regime design (standardized and individualised), length of treatment and level of direct observation of therapy (18).

We recommend implementation of universal rapid diagnostic tests for MDR (23) where there are adequate resources to pay for it as well as the immediate initiation of standardized MDR-TB treatment while awaiting DST results and elaboration of individualised regimens at the health facilities. Rapid molecular tests can speed up the detection of drug resistant TB, reduce the number of visits to the health facility for diagnosis and the number of suspects lost to follow up and may reduce overtreatment of smear negative TB in settings were clinical diagnosis is suboptimal (24). However, while the cost of implementing rapid molecular test may be prohibitive in resource-constrained settings, current evidence suggests they could become cost effective (25,26). A health technology assessment of the implementation of rapid molecular tests for drug resistant TB in addition to existing diagnostic tests (smear and phenotypic culture) in the United Kingdom, found them cost effective with Xpert MTB/RIF being the most cost effective. A modelling study that compared full roll out of Xpert MTB/RIF for all TB suspects in Tanzania to use only in smear negative TB suspects and in HIV positive TB suspects found the universal use of Xpert MTB/RIF to be the most cost effective (24). In the Peruvian context, where Genotype MTBRIF*plus*, MODS and GRIESS are implemented in addition to Löwenstein-Jensen, a new DST should only be added if it is more cost effective than an existing one and if the inferior test is replaced, for which a local health technology assessment should be conducted. Each DST has its own supply requirement, quality control system and training and supervision needs, thus it may be more efficient to have fewer DST.

National TB programmes in high and medium incidence settings such as Peru constantly make decisions concerning competing priorities. Correcting problems regarding the implementation of recommended strategies, such as reducing MDR-TB treatment initiation delay, testing for TB only persons at risk and improving supervisory practices would likely take priority over the implementation of new strategies. Resources should be sought for universalization of DST and for implementation of better performing DST. However, evidence-based, locally adapted criteria to select people at risk of MDR-TB and screening them, even with conventional tests in conjunction with swift initiation of

treatment, may contribute to reduction of transmission. Implementation of new strategies or new perspectives to existing protocols, such as extending household contact investigation requires further research.

4.4 Future research

The implications of the findings presented in this thesis call for a change in current policy and practice of TB and MDR-TB case detection. New hypotheses and suggestions for further research have also emerged from our findings. A thorough understanding of the case detection process and treatment initiation should complement research done on diagnostic tests.

Improving competence and work effectiveness of TB program staff and teams could positively impact the case detection process. All activities of the case detection process (selection of TB suspects, diagnosis, treatment initiation, contact investigation, supervision, data collection and interpretation) are conducted by individuals. Research on health services and on human resources could determine the profile of staff (training, attitude, skills) and the quantity, distribution, organization and managerial practices needed for diligent task conduction. The impact on case detection indicators could be measured.

Strong evidence supports implementing household contact investigation in all TB programs but in practice it is poorly conducted. Operational studies should determine the barriers to correct household contact investigation. Implementation studies should evaluate the efficiency of strategies that continue household contact follow-up to two years in order to match the highest epidemiological risk period. The role of incentives, health force training and patient and contact education could be considered within interventions. Mobile technology and e-health are a promising venue to be explored. Phone calls, text messages and other media could replace face-to-face contact between staff and household contacts while retaining TB risk awareness for a prolonged period, and are also potentially useful strategies that should be studied.

The recent progress made in the area of diagnostic tests made possible by the development of automated molecular tests would be galvanized when point-of-care testing for TB and drug resistant TB becomes available. However, the problems we identified will not be fully solved by a point-of-care test and the expected benefit and cost-effectiveness may not be attainable. Our findings support the need for health service research

addressing interventions to address gaps in laboratory management skills, leadership, supervision practices and quality control.

Modelling should determine the patterns and threshold of MDR-TB prevalence in given populations or geographical areas where universal DST is a more cost effective strategy in terms of reducing MDR-TB incidence than DST targeted to groups at risk. Empirical research will have to provide the necessary data for modelling studies. Despite the cost, in well-defined circumstances, additional efforts will have to be made to expand DST at an earlier stage of the epidemic.

The benefits of individualised regimens for MDR-TB treatment may well be offset by the complexities of implementing them correctly, while limitations of standardized regimens may be compensated by their easier implementation. Modelling could determine the impact of treatment initiation delay on transmission. Comparative studies in operational conditions should compare the effectiveness of using only standardized or individualised regimens or a combination of both. The marginal clinical benefit of individualising treatment may not be meaningful or may even be negative in less structured and understaffed TB programs. Research to determine the minimal conditions that general health services and TB programs need to fulfill in order to implement individualisation of drug resistant TB would provide valuable information for TB control.

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Curriculum vitae

Larissa Otero was born in Freiburg, Germany on January 26th 1980. She went to primary school in Geneva, Switzerland and then moved to Lima, Peru where she completed high school. She obtained a Medical Doctor degree at Universidad Peruana Cayetano Heredia (UPCH) in 2005. Her thesis was awarded the Peruvian Society of Infectious and Tropical Diseases and Induquímica Prize for HIV research. After some field experience in the Peruvian Andes and a first immersion in research in tuberculosis, she obtained a Master in Public Health with orientation in Disease Control and a mention on Tropical Disease at the Institute of Tropical Medicine in Antwerp, Belgium, in 2008. Her master thesis received the Prize for Development Cooperation of the Province of Antwerp. She worked with the non-governmental organization Médecins Sans Frontières in an HIV-TB program in Arua, Uganda and in an Ebola outbreak in Monrovia, Liberia. While based in Peru, she completed a fellowship in global health at University of California. She works as a researcher at the Instituto de Medicina Tropical Alexander von Humboldt at UPCH and holds a faculty appointment at the School of Medicine at UPCH.

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